(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 May 2001 (17.05.2001)

PCT

(10) International Publication Number
WO 01/34571 A1

(51) International Patent Classification[7]: C07D 223/18, 223/16, 243/12, 243/24, 267/14, 281/10, 487/04, A61K 31/5513, 31/5517, 31/551, 31/554, A61P 25/28 // (C07D 487/04, 243:00, 209:00)

(21) International Application Number: PCT/US00/26278

(22) International Filing Date: 26 October 2000 (26.10.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/164,399 9 November 1999 (09.11.1999) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

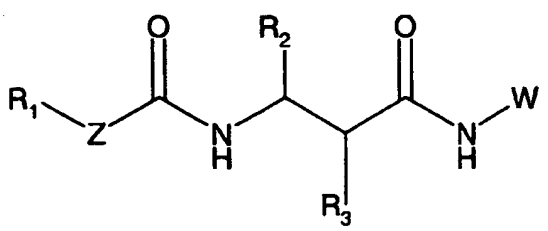
(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/34571 A1

(54) Title: β-AMINOACID COMPOUNDS USEFUL FOR INHIBITING β-AMYLOID PEPTIDE RELEASE AND/OR ITS SYNTHESIS



(I)

(57) Abstract: The present invention relates β-aminoacid containing compounds of formula (I) which inhibit β-amyloid peptide release and/or its synthesis and are useful in treating Alzheimer's disease and cognition enhancement.

β-AMINOACID COMPOUNDS USEFUL FOR INHIBITING β-AMYLOID PEPTIDE RELEASE AND/OR ITS SYNTHESIS

This invention relates to β-aminoacid containing compounds which inhibit β-amyloid peptide release and/or its synthesis and are useful in treating Alzheimer's disease.

BACKGROUND OF THE INVENTION

Alzheimer's Disease is a degenerative brain disorder characterized clinically by progressive loss of memory, cognition, reasoning, judgment and emotional stability that gradually leads to profound mental deterioration and ultimately death. Alzheimer's disease is a very common cause of progressive mental failure (dementia) in aged humans and is believed to represent the fourth most common medical cause of death in the United States. Alzheimer's disease has been observed in races and ethnic groups worldwide and presents a major present and future public health problem. The disease is currently estimated to affect about two to three million individuals in the United States alone. Alzheimer's disease is at present incurable. No treatment that effectively prevents Alzheimer's disease or reverses its symptoms and course is currently known.

The brains of individuals with Alzheimer's disease exhibit characteristic lesions termed senile (or amyloid) plaques, amyloid angiopathy (amyloid deposits in blood vessels) and neurofibrillary tangles. Large numbers of these lesions, particularly amyloid plaques and neurofibrillary tangles, are generally found in several areas of the human brain important for memory and cognitive function in patients with Alzheimer's disease. Smaller numbers of these lesions in a more restrictive anatomical distribution are also found in the brains of most aged humans who do not have clinical Alzheimer's disease. Amyloid plaques and amyloid angiopathy also characterize the brains of individuals with Trisomy 21 (Down's Syndrome) and

Hereditary Cerebral Hemorrhage with Amyloidosis of the Dutch Type (HCHWA-D). At present, a definitive diagnosis of Alzheimer's disease usually requires observing the aforementioned lesions in the brain tissue of patients who have died with the disease or, rarely, in small biopsied samples of brain tissue taken during an invasive neurosurgical procedure.

The principal chemical constituent of the amyloid plaques and vascular amyloid deposits (amyloid angiopathy) characteristic of Alzheimer's disease and the other disorders mentioned above is an approximately 4.2 kilodalton (kD) protein of about 39-43 amino acids designated the β-amyloid peptide (βAP) or sometimes Aβ, AβP or β/A4. β-Amyloid peptide was first purified and a partial amino acid sequence was provided by Glenner, et al. Biochem. Biophys. Res. Commun., **120**:885-890, (1984). The isolation procedure and the sequence data for the first 28 amino acids are described in U.S. Patent No. 4,666,8292.

Molecular biological and protein chemical analyzes have shown that the β-amyloid peptide is a small fragment of a much larger precursor protein termed the amyloid precursor protein (APP), that is normally produced by cells in many tissues of various animals, including humans. Knowledge of the structure of the gene encoding APP has demonstrated that β-amyloid peptide arises as a peptide fragment that is cleaved from APP by protease enzyme(s). The precise biochemical mechanism by which the β-amyloid peptide fragment is cleaved from APP and subsequently deposited as amyloid plaques in the cerebral tissue and in the walls of the cerebral and meningeal blood vessels is currently unknown.

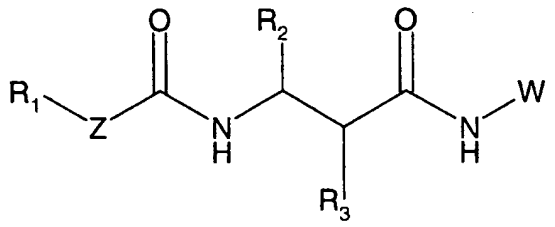
Several lines of evidence indicate that progressive cerebral deposition of β-amyloid peptide plays a seminal role in the pathogenesis of Alzheimer's disease and can precede cognitive symptoms by years or decades. See, for example, Selkoe, Neuron, **6**:487-498 (1991). The most

important line of evidence is the discovery that missense DNA mutations at amino acid 717 of the 770-amino acid isoform of APP can be found in affected members but not unaffected members of several families with a genetically determined (familial) form of Alzheimer's disease (Goate, et al., Nature, **349**:704-706 (1990); Chartier Harlan, et al., Nature, **353**:844-846 (1989); Murrell, et al., Science, **254**:97-99 (1991)) and is referred to as the Swedish variant. A double mutation changing lysine595-methionine596 to asparagine595-leucine596 (with reference to the 695 isoform) found in a Swedish family was reported in 1992 (Mullan, et al., Nature Genet., **1**:345-347 (1992)). Genetic linkage analyses have demonstrated that these mutations, as well as certain other mutations in the APP gene, are the specific molecular cause of Alzheimer's disease in the affected members of such families. In addition, a mutation at amino acid 693 of the 770-amino acid isoform of APP has been identified as the cause of the β-amyloid peptide deposition disease, HCHWA-D, and a change from alanine to glycine at amino acid 692 appears to cause a phenotype that resembles Alzheimer's disease is some patients but HCHWA-D in others. The discovery of these and other mutations in APP in genetically based cases of Alzheimer's disease prove that alteration of APP and subsequent deposition of its β-amyloid peptide fragment can cause Alzheimer's disease.

Despite the progress which has been made in understanding the underlying mechanisms of Alzheimer's disease and other β-amyloid peptide related diseases, there remains a need to develop methods and compositions for treatment of the disease(s). Ideally, the treatment methods would advantageously be based on drugs which are capable of inhibiting β-amyloid peptide release and/or its synthesis in vivo.

SUMMARY OF THE INVENTION

This invention provides β-aminoacid containing compounds of formula I:



formula I

wherein

R_1 is selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, aryl, heteroaryl and heterocyclic;

R_2 is selected from the group consisting of hydrogen, alkyl, cycloalkyl, and aryl;

R_3 is selected from the group consisting of hydrogen, alkyl, cycloalkyl, and aryl;

Z is represented by the formula -CX'X"-

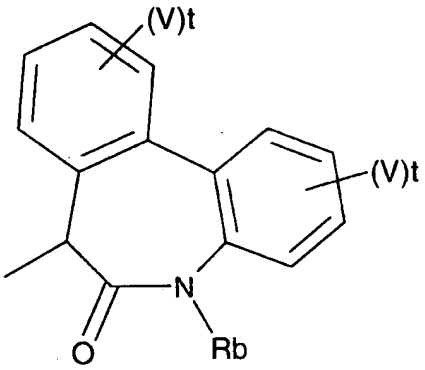
wherein

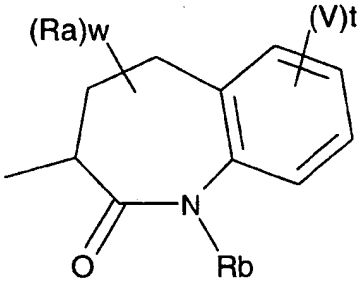
X' is selected from the group consisting of hydrogen, hydroxy, and fluoro,

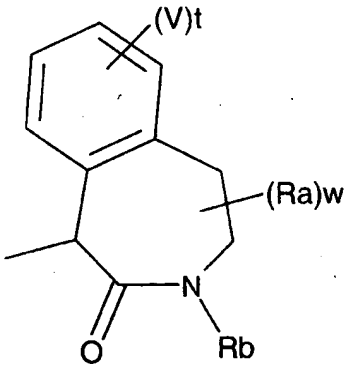
X" is selected from the group consisting of hydrogen, hydroxy, and fluoro, or

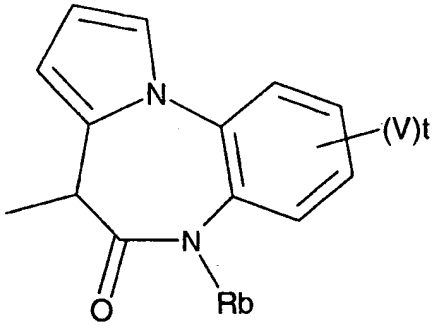
X' and X" together form an oxo group;

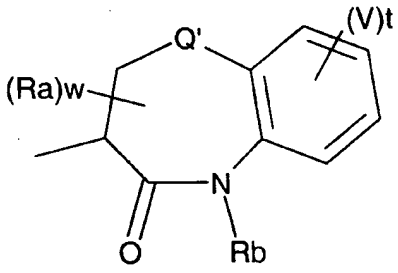
W is a cyclic group selected from the group consisting of

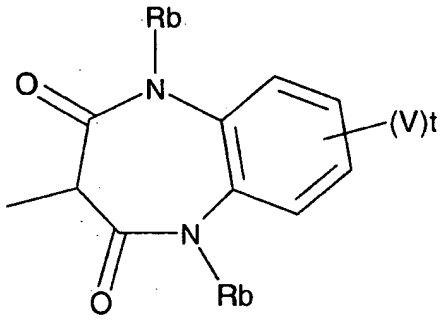

(V)t
(V)t
N
O
Rb

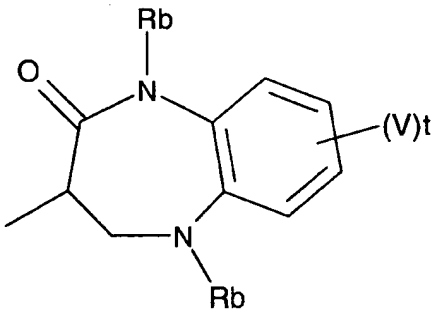

(Ra)w
(V)t
N
O
Rb

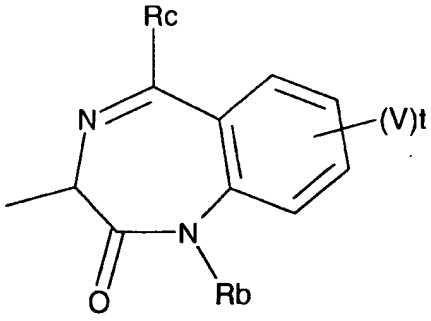

(V)t
(Ra)w
N
O
Rb


N
(V)t
N
O
Rb


(V)t
Q'
(Ra)w
N
O
Rb


Rb
N
O
(V)t
N
O
Rb


Rb
O
N
(V)t
N
Rb


Rc
N
(V)t
N
O
Rb

wherein
Q' is oxygen or sulfur;

each V is independently selected from the group consisting of hydroxy, acyl, acyloxy, alkyl, substituted alkyl, alkoxy, substituted alkoxy, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, amino, aminoacyl, alkaryl, aryl, aryloxy, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, thioalkoxy, substituted thioalkoxy, trihalomethyl;

Ra is selected from the group consisting of alkyl, substituted alkyl, alkoxy, substituted alkoxy, amino, carboxyl, carboxyl alkyl, cyano, halo;

Rb is selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, acyl, aryl, heteroaryl, heterocyclic;

Rc is selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, heteroaryl, heterocyclic, cycloalkyl, and substituted cycloalkyl;

t is an integer from 0 to 4;

w is an integer from 0 to 4;

and the pharmaceutically acceptable salts thereof.

This invention also provides for novel pharmaceutical compositions comprising a compound of the formula I and a pharmaceutically acceptable diluent.

Additionally, this invention provides a method for inhibiting β-amyloid peptide release and/or its synthesis in a cell which method comprises administering to such a cell an amount of a compound or a mixture of compounds of formula I above effective in inhibiting the cellular release and/or synthesis of β-amyloid peptide.

Because the in vivo generation of β-amyloid peptide is associated with the pathogenesis of Alzheimer's disease the compounds of formula I can also be employed in conjunction with a pharmaceutical composition to prophylactically and/or therapeutically prevent and/or treat Alzheimer's disease. Accordingly, the present invention provides a prophylactic method for preventing the onset of Alzheimer's disease in a patient at risk for developing Alzheimer's disease which method comprises administering to said patient a pharmaceutical composition comprising a pharmaceutically inert carrier and an effective amount of a compound or a mixture of compounds of formula I above.

The present invention also provides a therapeutic method for treating a patient with Alzheimer's disease in order to inhibit further deterioration in the condition of that patient which method comprises administering to said patient a pharmaceutical composition comprising a pharmaceutically inert carrier and an effective amount of a compound or a mixture of compounds of formula I above.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the following terms have the meanings indicated:

The term "β-amyloid peptide" refers to a 39-43 amino acid peptide having a molecular weight of about 4.2 kD, which peptide is substantially homologous to the form of the protein described by Glenner, et al. Biochem. Biophys. Res. Commun. (1984) **120**:885-890, including mutations and post-translational modifications of the normal β-amyloid peptide. In whatever form, the β-amyloid peptide is an approximate

39-43 amino acid fragment of a large membrane-spanning glycoprotein, referred to as the β-amyloid precursor protein (APP). Its 43-amino acid sequence is:

1
Asp Ala Glu Phe Arg His Asp Ser Gly Tyr
11
Glu Val His His Gln Lys Leu Val Phe Phe
21
Ala Glu Asp Val Gly Ser Asn Lys Gly Ala
31
Ile Ile Gly Leu Met Val Gly Gly Val Val
41
Ile Ala Thr (SEQ ID NO: 1)

or a sequence which is substantially homologous thereto.

"Alkyl" refers to monovalent alkyl groups preferably having from 1 to 20 carbon atoms and more preferably 1 to 6 carbon atoms. This term is exemplified by groups such as methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, n-pentyl, n-hexyl, and the like. It is understood that the term alkyl includes C_1-C_4 alkyl.

"C_1-C_4 alkyl" refers to monovalent alkyl groups preferably having from 1 to 4 carbon atoms. This term is exemplified by groups such as methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, and t-butyl.

"Substituted alkyl" refers to an alkyl group, preferably of from 1 to 10 carbon atoms, having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyacylamino, cyano, halogen, hydroxyl, carboxyl, carboxylalkyl, keto, thioketo, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic,

heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl,-SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl and -SO_2-heteroaryl.

"Alkenylene" refers to divalent alkenylene groups preferably having from 2 to 10 carbon atoms and more preferably 2 to 6 carbon atoms. This term is exemplified by groups such as ethenylene (-CH=CH-), the propenylene isomers (e.g., -CH_2CH=CH- and -C(CH_3)=CH-) and the like.

"Substituted alkenylene" refers to an alkenylene group, preferably of from 2 to 10 carbon atoms, having from 1 to 3 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkoxy, substituted cycloalkoxyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, cyano, halogen, hydroxyl, carboxyl, carboxylalkyl, keto, thioketo, thiol, thioalkoxy, substituted thioalkoxy, aryl, heteroaryl, heterocyclic, heterocyclooxy, nitro -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl, and -SO_2-heteroaryl. Additionally, such substituted alkylene groups include those where 2 substituents on the alkylene group are fused to form one or more cycloalkyl, aryl, heterocyclic or heteroaryl groups fused to the alkylene group.

"Alkaryl" refers to -alkylene-aryl groups preferably having from 1 to 8 carbon atoms in the alkylene moiety and from 6 to 10 carbon atoms in the aryl moiety. Such alkaryl groups are exemplified by benzyl, phenethyl and the like.

"Alkoxy" refers to the group "alkyl-O-". Preferred alkoxy groups include, by way of example, methoxy, ethoxy, n-propoxy, iso-propoxy, n-butoxy, tert-butoxy, sec-butoxy, n-pentoxy, n-hexoxy, 1,2-dimethylbutoxy, and the like.

"Substituted alkoxy" refers to the group "substituted alkyl-O-" where substituted alkyl is as defined above.

"Alkenyl" refers to alkenyl groups preferably having from 2 to 10 carbon atoms and more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-2 sites of

alkenyl unsaturation. Preferred alkenyl groups include ethenyl(-CH=CH_2), n-propenyl (-CH_2CH=CH_2), iso-propenyl (-C(CH_3)=CH_2), and the like.

"Substituted alkenyl" refers to an alkenyl group as defined above having from 1 to 3 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkoxy, substituted cycloalkoxyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, cyano, halogen, hydroxyl, carboxyl, carboxylalkyl, keto, thioketo, thiol, thioalkoxy, substituted thioalkoxy, aryl, heteroaryl, heterocyclic, heterocyclooxy, nitro -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl, and -SO_2-heteroaryl.

"Alkynyl" refers to alkynyl groups preferably having from 2 to 10 carbon atoms and more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-2 sites of alkynyl unsaturation. Preferred alkynyl groups include ethynyl, propargyl, and the like.

"Substituted alkynyl" refers to an alkynyl group as defined above having from 1 to 3 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkoxy, substituted cycloalkoxyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, cyano, halogen, hydroxyl, carboxyl, carboxylalkyl, keto, thioketo, thiol, thioalkoxy, substituted thioalkoxy, aryl, heteroaryl, heterocyclic, heterocyclooxy, nitro -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl, and -SO_2-heteroaryl.

"Acyl" refers to the groups alkyl-C(O)-, substituted alkyl-C(O)-, cycloalkyl-C(O)-, substituted cycloalkyl-C(O)-, aryl-C(O)-, heteroaryl-C(O)- and heterocyclic-C(O)- where alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, aryl, heteroaryl and heterocyclic are as defined herein.

"Acylamino" refers to the group -C(O)NRR where each R is independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, heterocyclic and where both R groups are joined to form a heterocyclic group, wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

"Substituted amino" refers to the group $-N(R)_2$ where each R is independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, aryl, cycloalkyl, substituted cycloalkyl, and where both R groups are joined to form a heterocyclic group. As is readily apparent to those skilled in the art, when both R groups are hydrogen, $-N(R)_2$ is an amino group. Examples of substituted amino groups include, by way of illustration, mono- and di-alkylamino, mono- and di-(substituted alkyl)amino, mono- and di-arylamino, mono- and di-heteroarylamino, mono- and di-heterocyclic amino, and unsymmetric di-substituted amines having different substituents selected from alkyl, substituted alkyl, aryl, and the like.

The term "blocking group" or "protecting group" refers to any group which prevents undesired reactions from occurring at the protected functionality and which may be removed by conventional chemical and/or enzymatic procedures. Selection and use of protecting groups is well understood and appreciated in the art. For example see, Protecting Groups in Organic Synthesis, Theodora Greene (1^{st} and 2^{nd} Editions, Wiley-Interscience). A protecting group may also be a covalently attached to a solid support as is well known and appreciated in the art of peptide synthesis and combinatorial chemistry.

"Aminoacyl" refers to the group -NRC(O)R where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

"Aminoacyloxy" refers to the group -NRC(O)OR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

"Acyloxy" refers to the groups alkyl-C(O)O-, substituted alkyl-C(O)O-, cycloalkyl-C(O)O-, substituted cycloalkyl-C(O)-, aryl-C(O)O-, heteroaryl-C(O)O-, and heterocyclic-C(O)O- wherein alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, aryl, heteroaryl, and heterocyclic are as defined herein.

"Aryl" refers to an unsaturated aromatic carbocyclic group of from 6 to 14 carbon atoms having a single ring (e.g., phenyl) or multiple condensed (fused) rings (e.g., naphthyl or anthryl). Preferred aryls include phenyl, naphthyl and the like.

Unless otherwise constrained by the definition for the aryl substituent, such aryl groups can optionally be substituted with from 1 to 5 substituents selected from the group consisting of acyloxy, hydroxy, acyl, alkyl, alkoxy, alkenyl, alkynyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, amino, substituted amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, heterocyclic, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl, $-SO_2$-heteroaryl and trihalomethyl. Preferred substituents include alkyl, alkoxy, halo, cyano, nitro, trihalomethyl, and thioalkoxy.

"Aryloxy" refers to the group aryl-O- wherein the aryl group is as defined above including optionally substituted aryl groups as also defined above.

"Carboxyalkyl" refers to the groups "-C(O)Oalkyl" and "-C(O)O-substituted alkyl" where alkyl is as defined above.

"Cycloalkyl" refers to cyclic alkyl groups of from 3 to 12 carbon atoms having a single cyclic ring or multiple condensed rings. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, and the like, or multiple ring structures such as quininclidine, adamantanyl, and the like.

"Substituted cycloalkyl" refers to cycloalkyl groups having from 1 to 5 (preferably 1 to 3) substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyacylamino, cyano, halogen, hydroxyl, carboxyl, carboxylalkyl, keto, thioketo, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl, and $-SO_2$-heteroaryl.

"Cycloalkenyl" refers to cyclic alkenyl groups of from 4 to 8 carbon atoms having a single cyclic ring and at least one point of internal unsaturation. Examples of suitable cycloalkenyl groups include, for instance, cyclobut-2-enyl, cyclopent-3-enyl, cyclooct-3-enyl and the like.

"Substituted cycloalkenyl" refers to cycloalkenyl groups having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyacylamino, cyano, halogen, hydroxyl, carboxyl, carboxylalkyl, keto, thioketo, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl, and $-SO_2$-heteroaryl.

"Halo" or "halogen" refers to fluoro, chloro, bromo and iodo and preferably is either fluoro or chloro.

"Heteroaryl" refers to an aromatic group of from 1 to 15 carbon atoms and 1 to 4 heteroatoms selected from oxygen, nitrogen and sulfur within at least one ring (if there is more than one ring).

Unless otherwise constrained by the definition for the heteroaryl substituent, such heteroaryl groups can be optionally substituted with 1 to 5 substituents selected from the group consisting of acyloxy, hydroxy, acyl, alkyl, alkoxy, alkenyl, alkynyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, amino, substituted amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, heterocyclic, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl, $-SO_2$-heteroaryl and trihalomethyl. Such heteroaryl groups can have a single ring (e.g., pyridyl or furyl) or multiple condensed rings (e.g., indolizinyl or benzothienyl). Preferred heteroaryls include pyridyl, pyrrolyl and furyl.

"Heteroaryloxy" refers to the group "-O-heteroaryl".

"Heterocycle" or "heterocyclic" refers to a monovalent saturated or unsaturated group having a single ring or multiple condensed rings, from 1 to 15 carbon atoms and from 1 to 4 hetero atoms selected from nitrogen, sulfur or oxygen within the ring.

Unless otherwise constrained by the definition for the heterocyclic substituent, such heterocyclic groups can be optionally substituted with 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyacylamino,

cyano, halogen, hydroxyl, carboxyl, carboxylalkyl, keto, thioketo, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl, and $-SO_2$-heteroaryl. Such heterocyclic groups can have a single ring or multiple condensed rings. Preferred heterocyclics include morpholino, piperidinyl, and the like.

Examples of heterocycles and heteroaryls include, but are not limited to, pyrrole, furan, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, morpholino, piperidinyl, tetrahydrofuranyl, and the like as well as N-alkoxy-nitrogen containing heterocycles.

"Heterocyclooxy" refers to the group "-O-heterocycle".

"Oxyacylamino" refers to the group -OC(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

"Thioalkoxy" refers to the group -S-alkyl.

"Substituted thioalkoxy" refers to the group -S-substituted alkyl.

"Thioaryloxy" refers to the group aryl-S- wherein the aryl group is as defined above including optionally substituted aryl groups also defined above.

"Thioheteroaryloxy" refers to the group heteroaryl-S- wherein the heteroaryl group is as defined above including optionally substituted aryl groups as also defined above.

The term "pharmaceutically-acceptable addition salt" refers to an acid addition salt.

The compound of formula I and the intermediates described herein form pharmaceutically acceptable acid addition salts with a wide variety of organic and inorganic acids and include the physiologically acceptable salts which are often used in pharmaceutical chemistry. Such salts are also part of this invention. A pharmaceutically-acceptable addition salt is formed from a pharmaceutically-acceptable acid as is well known in the art. Such salts include the pharmaceutically acceptable salts listed in Journal of Pharmaceutical Science, 66, 2-19 (1977) which are known to the skilled artisan. Typical inorganic acids used to form such salts include hydrochloric, hydrobromic, hydriodic, nitric, sulfuric, phosphoric, hypophosphoric, metaphosphoric, pyrophosphoric, and the like. Salts derived from organic acids, such as aliphatic mono and dicarboxylic acids, phenyl substituted alkanoic acids, hydroxyalkanoic and hydroxyalkandioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, may also be used. Such pharmaceutically acceptable salts thus include acetate, phenylacetate, trifluoroacetate, acrylate, ascorbate, benzoate, chlorobenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, methylbenzoate, o-acetoxybenzoate, naphthalene-2-benzoate, bromide, isobutyrate, phenylbutyrate, α-hydroxybutyrate, butyne-1,4-dicarboxylate, hexyne-1,4-dicarboxylate, caprate, caprylate, cinnamate, citrate, formate, fumarate, glycollate, heptanoate, hippurate, lactate, malate, maleate, hydroxymaleate, malonate, mandelate, mesylate, nicotinate, isonicotinate, nitrate, oxalate, phthalate, teraphthalate, propiolate, propionate, phenylpropionate, salicylate, sebacate, succinate, suberate, benzenesulfonate, p-bromobenzenesulfonate, chlorobenzenesulfonate, ethanesulfonate, 2-hydroxyethanesulfonate, methanesulfonate,

naphthalene-1-sulfonate, naphthalene-2-sulfonate, p-toluenesulfonate, xylenesulfonate, tartarate, and the like.

The term "ee" or "enantiomeric excess" refers to the percent by which one enantiomer, E1, is in excess in a mixture of both enantiomers (E1 + E2), as calculated by the equation {(E1 - E2) ÷ (E1 + E2)} x 100% = ee.

As is appreciated by the skilled person, compounds of formula I exist as stereoisomers. The present invention relates to the stereoisomers of the compounds of formula I. Herein, the Cahn-Prelog-Ingold designations of (R)- and (S)- and the designations of L- and D- for stereochemistry relative to the isomers of glyceraldehyde are used to refer to specific isomers where designated.

The specific isomers of the compounds of formula I can by prepared by stereospecific synthesis. The compounds of formula I and the starting materials for their preparation can be resolved and recovered by techniques known in the art, such as, chromatography on chiral stationary phases, and fractional recrystallization of addition salts formed by reagents used for that purpose. Useful methods of resolving and recovering specific stereoisomers are known in the art and described in Stereochemistry of Organic Compounds, E.L. Eliel and S.H. Wilen (Wiley-Interscience 1994), Enantiomers, Racemates, and Resolutions, J. Jacques, A. Collet, and S.H. Wilen (Wiley-Interscience 1981), and European Patent Application No. EP-A-838448, published April 29, 1998.

It is to be understood that the invention extends to each of the isomeric forms of the compounds of the present invention including the geometric, diastereomeric, enantiomeric, and racemic forms of the compound of formula I.

As with any group of pharmaceutically active compounds, some groups are preferred in their end use application. Preferred embodiments of the present invention are given below:

Compounds in which R_1 is alkyl or aryl are preferred.

Compounds in which R_1 is C_1-C_4 alkyl are more preferred, with isopropyl being most preferred.

Compounds in which R_1 is phenyl substituted with from 1 to 3 substituents selected from the group consisting of hydrogen, alkyl, alkoxy, and halo are more preferred with 3,5-difluorophenyl being most preferred.

Compounds in which R_2 and R_3 are hydrogen, alkyl, cycloalkyly, or aryl are preferred.

Compounds in which one of R_2 or R_3 are hydrogen are preferred.

Compounds in which R_2 or R_3 are C_1-C_4 alkyl are more preferred, with methyl being most preferred.

Compounds in which R_2 or R_3 are phenyl substituted with from 1 to 3 substituents selected from the group consisting of hydrogen, alkyl, alkoxy, and halo are more preferred with phenyl being most preferred.

Compounds in which Z is $-CH_2-$ or $-CH(OH)-$ are preferred.

Compounds in which V is alkyl, aryl, or halo are preferred.

Compounds in which Ra is alkyl or halo are preferred.

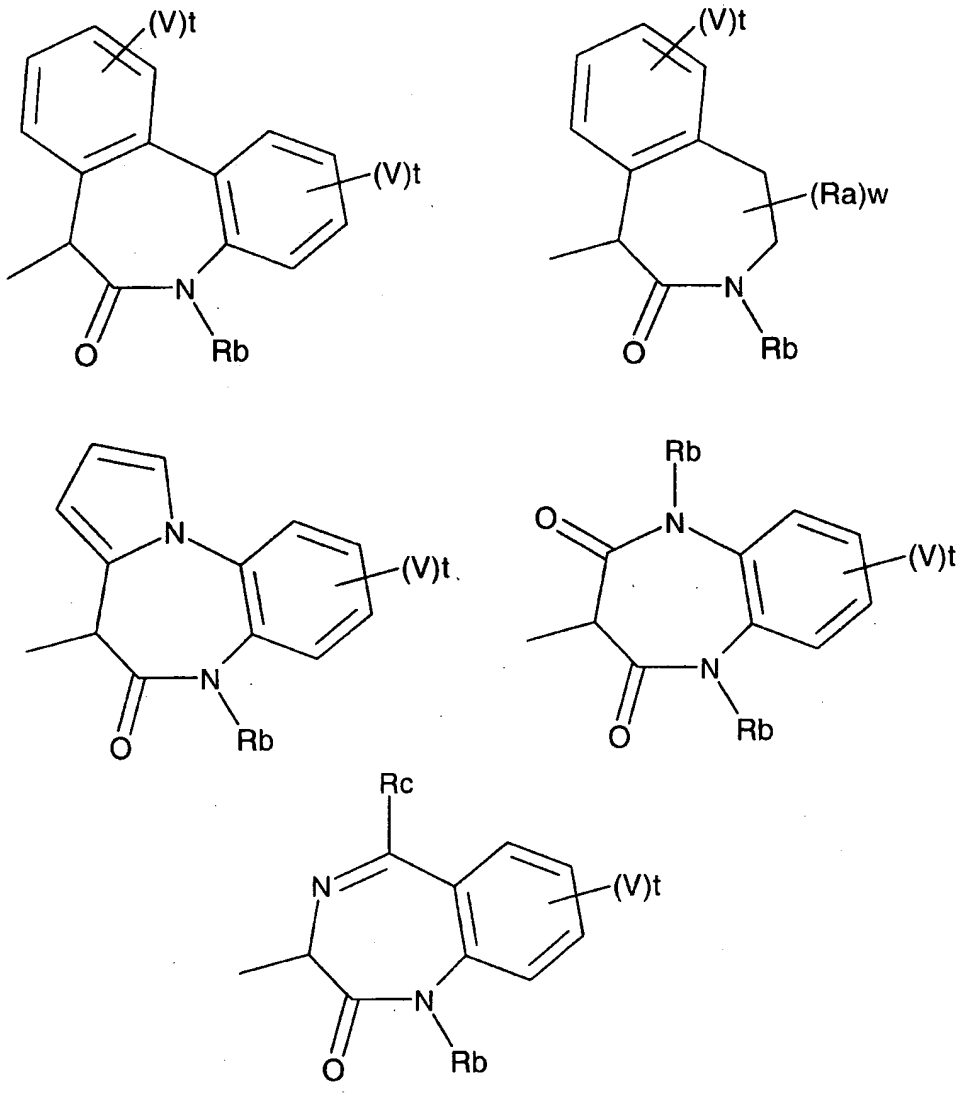
Compounds in which Rb is hydrogen, alkyl, or aryl are preferred.

Compounds in which Rc is alkyl or aryl are preferred.

Compounds in which t is 0 are preferred.

Compounds in which w is 0 are preferred.

Compounds in which W is a cyclic group selected from the group consisting of



wherein

Rb is selected from the group consisting of hydrogen, alkyl and aryl;

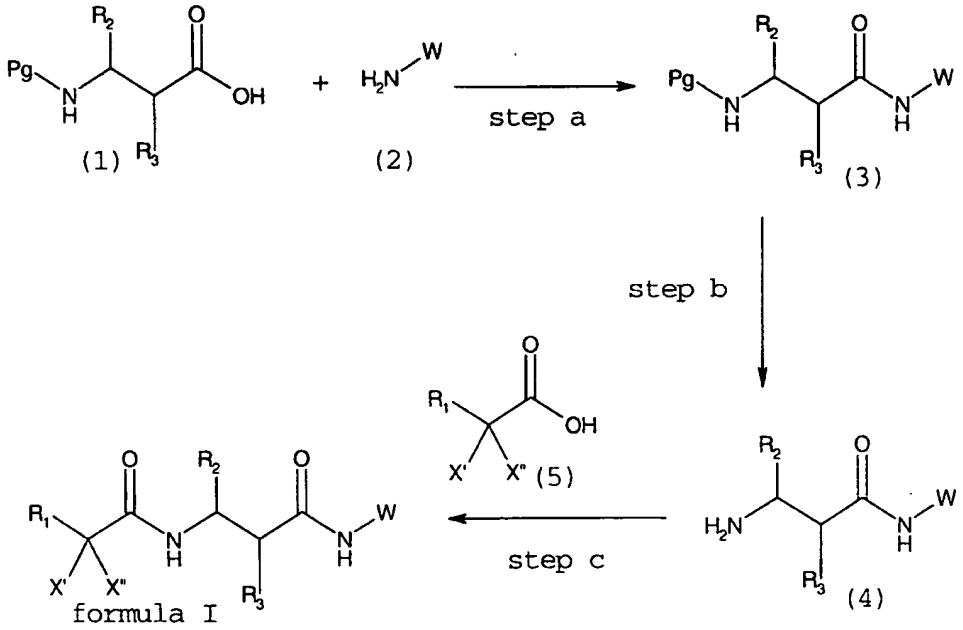
Rc is selected from the group consisting of alkyl, and aryl;

t is 0; and

w is 0; are more preferred.

The compounds of formula I are prepared as described in Reaction Scheme A.1 and A.2 below. Reaction Scheme A.1 and A.2, all substituents, unless otherwise indicated, are as previously defined. Reaction Scheme A.1 and A.2 all reagents are well known and appreciated in the art.

Reaction Scheme A.1



Reaction Scheme A.1, step a, depicts the coupling reaction of an appropriate amino-protected β-amino acid of formula (1) and an appropriate compound W-NH_2 of formula

(2). Appropriate amino-protected β-amino acids are ones in which R_2 and R_3 are as desired in the final product of formula I and readily available to the person skilled in the art and can be prepared as described herein. An appropriate compound of formula (1) may also have the stereochemistry that is desired in the final compound of formula I. An appropriate compound of formula (2) is one in which W is as desired in the final compound of formula I. An appropriate compound of formula (2) may also have the stereochemistry that is desired in the final compound of formula I. Compounds of formula (2) are also readily available to the skilled person as described in PCT Application No. PCT/US97/22986, filed 22 December 1997, and our co-pending application USSN 09/163873, filed September 30, 1999, the disclosures of which are incorporated herein by reference in their entirety.

Additionally, the synthesis of various benzazepinones and related compounds are described in Busacca et al., Tetrahedron Lett., 33, 165-168 (1992); Crosisier et al., U.S. Patent No. 4,080,449; J. A. Robl et al. Tetrahedron Lett., 36(10), 1593-1596 (1995); Flynn et al. J. Med. Chem. 36, 2420-2423 (1993); Orito et al. Tetrahedron, 36, 1017-1021 (1980); Kawase et al., J. Org. Chem., 54, 3394-3403 (1989); Lowe et al., J. Med. Chem. 37, 3789-3811 (1994); Robl et al., Bioorg. Med. Chem. Lett., 4, 1789-1794 (1994); Skiles et al., Bioorg. Med. Chem. Lett., 3, 773-778 (1993); Grunewald et al., J. Med. Chem., 39(18), 3539- (1996); Warshawsky et al., Bioorg. Med. Chem. Lett., 6, 957-962 (1996); Ben-Ishai, et al., Tetrahedron, 43, 439-450 (1987); van Neil et al, Bioorg. Med. Chem. 5, 1421-1426 (1995); and reference cited therein. These publications and patents are incorporated herein by reference in their entirety.

Similarly, various benzodiazepine derivatives suitable for use in this invention can be prepared using conventional procedures and reagents. For example, a 2-aminobenzophenone can be readily coupled to α-(isopropylthio)-N-

(benzyloxycarbonyl)glycine by first forming the acid chloride of the glycine derivative with oxayl chloride, and then coupling the acid chloride with the 2-aminobenzophenone in the presence of a base, such as 4-methylmorpholine, to afford the 2-(α-(isopropylthio)-N-(benzyloxycarbonyl) glycinyl)-aminobenzophenone. Treatment of this compound with ammonia gas in the presence of an excess, preferably about 1.1 to about 1.5 equivalents, of mercury (II) chloride then affords the 2-(N-(α-amino)-N'-(benzyloxycarbonyl)-glycinyl)aminobenzophenone. This intermediate can then be readily cyclized by treatment with glacial acetic acid and ammonium acetate to provide the 3-(benzyloxycarbonyl)amino-2,3-dihydro-5-phenyl-1H-1,4-benzodiazepin-2-one. Subsequent removal of the Cbz group affords the 3-amino-2,3-dihydro-5-phenyl-1H-1,4-benzodiazepin-2-one.

Alternatively, 2,3-dihydro-5-phenyl-1H-1,4-benzodiazepin-2-ones can be readily aminated at the 3-position using conventional azide transfer reactions followed by reduction of the resulting azido group to form the corresponding amino group. The conditions for these and related reactions are described in the examples set forth below. Additionally, 2,3-dihydro-5-phenyl-1H-1,4-benzodiazepin-2-ones are readily alkylated at the 1-position using conventional procedures and reagents. For example, this reaction is typically conducted by first treating the benzodiazepinone with about 1.1 to about 1.5 equivalents of a base, such as sodium hydride, potassium tert-butoxide, potassium 1,1,1,3,3,3-hexamethyldisilazane, cesium carbonate, in an inert diluent, such as DMF. This reaction is typically conducted at a temperature ranging from about -78ºC to about 80ºC for about 0.5 to about 6 hours. The resulting anion is then contacted with an excess, preferably about 1.1 to about 3.0 equivalents, of an alkyl halide, typically an alkyl chloride, bromide or iodide. Generally, this reaction is conducted at a temperature of about 0ºC to about 100ºC for about 1 to about 48 hours. Additionally, the

3-amino-2,4-dioxo-2,3,4,5-tetrahydro-1H-1,5-benzodiazepines employed in this invention are typically prepared by first coupling malonic acid with a 1,2-phenylenediamine. Conditions for this reaction are well known in the art and are described, for example, in PCT Application WO 96-US8400 960603. Subsequent alkylation and amination using conventional procedures and reagents affords various 3-amino-1,5-bis(alkyl)-2,4-dioxo-2,3,4,5-tetrahydro-1H-1,5-benzodiazepines. Such procedures are described in detail in PCT Application No. PCT/US97/22986.

The coupling reaction depicted in Reaction Scheme A.1, step a, involves a reaction which is conventionally conducted for peptide synthesis and synthetic methods used therein can also be employed. For example, well known coupling reagents such as carbodiimides with or without the use of well known additives such as N-hydroxysuccinimide, 1-hydroxybenzotriazole, etc. can be used to facilitate coupling. The reaction is conventionally conducted in an inert aprotic polar diluent such as dimethylformamide, methylene chloride, chloroform, acetonitrile, tetrahydrofuran and the like. Alternatively, the acid halide of compound (1) can be employed in the reaction and, when so employed, it is typically employed in the presence of a suitable base to scavenge the acid generated during the reaction. Suitable bases include, by way of example, triethylamine, N,N-diisopropylethylamine, N-methylmorpholine and the like.

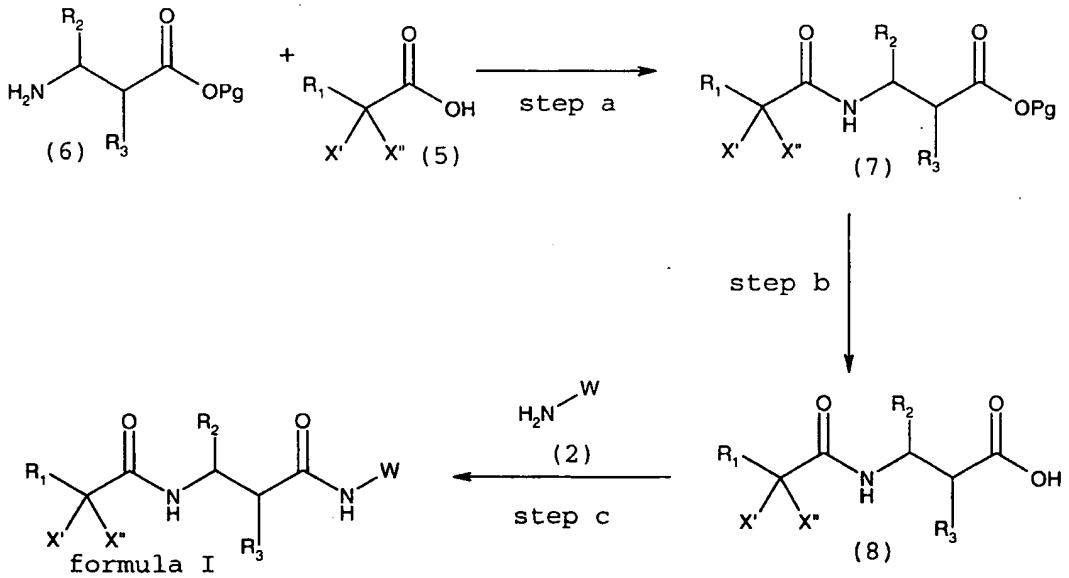
The reaction is preferably conducted at from about 0ºC to about 60ºC until reaction completion which typically occurs within 1 to about 24 hours. Upon reaction completion, the product of formula (3) is recovered by conventional methods including precipitation, chromatography, filtration and the like or alternatively is deprotected to the corresponding amine of formula (4) without purification and/or isolation other than conventional work-up (e.g., aqueous extraction, etc.).

Reaction Scheme A.1, step b, depicts the deprotection of a compound of formula (3) to give a compound or formula (4). Such deprotections of amino protecting groups is well known and appreciated in the art.

Reaction Scheme A.1, step c, depicts the coupling reaction of an appropriate compound of formula (5), $R_1CX'X''C(O)$-OH, and a compound of formula (4). Appropriate compounds of formula (5) are compounds in which R_1, X' and X" are as desired in the final product of formula I and are well known in the art and available as described herein. An appropriate compound of formula (5) may also have the stereochemistry that is desired in the final compound of formula I. The coupling reaction depicted in step c is carried out using the acid of formula (5) or the acid halide derived therefrom, in a manner similar to those taught in Reaction Scheme A.1, step a.

Reaction Scheme A.1, optional step d, not shown, an acid addition salt is formed using a pharmaceutically-acceptable acid. The formation of acid addition salts is well known and appreciated in the art.

Reaction Scheme A.2



Reaction Scheme A.2, step a, depicts the coupling reaction of an appropriate carboxy-protected β-amino acid of formula (6) and an appropriate compound of formula (5), as described above, to give a compound of formula (7). Appropriate carboxy-protected β-amino acids are ones in which R_2 and R_3 are as desired in the final product of formula I and readily available to the person skilled in the art and can be prepared as described herein. An appropriate compound of formula (6) may also have the stereochemistry that is desired in the final compound of formula I. This coupling reaction is carried out using the acid of formula (5) or the acid halide derived therefrom, in a manner similar to those taught in Reaction Scheme A.1, step a.

Reaction Scheme A.2, step b, depicts the deprotection of a compound of formula (7) to give a compound or formula (8). Such deprotections of carboxy protecting groups is well known and appreciated in the art.

Reaction Scheme A.2, step c, depicts the coupling reaction of an appropriate compound of formula (2), as described above, and a compound of formula (8). Appropriate compounds of formula (2) are the coupling reaction depicted in step c are taught in Reaction Scheme A.1, step a.

Reaction Scheme A.2, optional step d, not shown, an acid addition salt is formed using a pharmaceutically-acceptable acid. The formation of acid addition salts is well known and appreciated in the art.

The following synthetic and biological examples are offered to illustrate this invention and are not to be construed in any way as limiting the scope of this invention.

In the examples below, the following abbreviations have the following meanings. If an abbreviation is not defined, it has its generally accepted meaning.

BEMP refers to 2-tert-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine; Boc refers to t-butoxycarbonyl; BOP refers to benzotriazol-1-yloxy-

tris(dimethylamino)phosphonium hexafluorophosphate; bd refers to broad doublet; bs refers to broad singlet; d refers to doublet; dd refers to doublet of doublets; DIC refers to diisopropyl carbodiimide; DMF refers to dimethylformamide; DMAP refers to 4-dimethylaminopyridine; DMSO refers to dimethylsulfoxide; EDC refers to ethyl-1-(3-dimethyaminopropyl)carbodiimide; eq. or eqv. refer to equivalents; EtOAc refers to ethyl acetate; g refers to grams; h refers to hours; HOBT refers to 1-hydroxybenzotriazole hydrate; Hunig's base refers to N,N-diisopropylethylamine; L refers to liter; m refers to multiplet; M refers to molar; max refers to maximum; meq refers to milliequivalent; mg refers to milligram; mL refers to milliliter; mm refers to millimeter; mmol refers to millimole; MOC refers to methoxyoxycarbonyl; N refers to normal; N/A refers to not available; ng refers to nanogram; nm refers to nanometers; OD refers to optical density; PEPC refers to 1-(3-(1-pyrrolidinyl)propyl)-3-ethylcarbodiimide; PP-HOBT refers to piperidine-piperidine-1-hydroxybenzotrizole; psi refers to pounds per square inch; Ph refers to phenyl; q refers to quartet; quint. refers to quintet; rpm refers to rotations per minute; s refers to singlet; t refers to triplet; TFA refers to trifluoroacetic acid; THF refers to tetrahydrofuran; tlc refers to thin layer chromatography; μL refers to microliter; UV refers to ultra-violet.

In the examples below, all temperatures are in degrees Celsius (unless otherwise indicated). The compounds set forth in the examples below were prepared using the following general procedures as indicated.

GENERAL PROCEDURE A

First EDC Coupling Procedure

To a 1:1 mixture of the corresponding acid and the corresponding amine in methylene chloride at 0°C was added 1.5 equivalents triethylamine, followed by 2.0 equivalents hydroxybenzotriazole monohydrate and then 1.25 equivalents of ethyl-3-(3-dimethylamino)propyl carbodiimide · HCl. The reaction mixture was stirred overnight at room temperature and then transferred to a separatory funnel. The mixture was washed with water, saturated aqueous $NaHCO_3$, 1N HCl and saturated aqueous NaCl, and then dried over $MgSO_4$. The resulting solution was stripped free of solvent on a rotary evaporator to yield the crude product.

GENERAL PROCEDURE B

Second EDC Coupling Procedure

A mixture of the corresponding acid (1 eqv), N-1-hydroxybenzotriazole (1.6 eqv), the corresponding amine (1 eqv), N-methylmorpholine (3 eqv) and methylene chloride (or DMF for insoluble substrates) was cooled in an ice-water bath and stirred until a clear solution was obtained. EDC (1.3 eqv) was then added to the reaction mixture. The cooling bath was then allowed to warm to ambient temperature over 1-2 h and the reaction mixture was stirred overnight. The reaction mixture was then evaporated to dryness under vacuum. To the residue was added 20% aqueous potassium carbonate and the mixture was shaken thoroughly and then allowed to stand until the oily product solidified (overnight if necessary). The solid product was then collected by filtration, washed thoroughly with 20% aqueous potassium carbonate, water, 10% HCl, and water to give the product, usually in pure state. No racemization was observed.

GENERAL PROCEDURE C

Third EDC Coupling Procedure

The acid was dissolved in methylene chloride. The amine (1 eq.), N-methylmorpholine (5 eq.) and hydroxybenzotriazole monohydrate (1.2 eq.) were added in sequence. A cooling bath was applied to the round bottomed flask until the solution reached 0°C. At that time, 1.2 eq. of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride was added. The solution was allowed to stir overnight and come to room temperature under nitrogen pressure. The reaction mixture was worked up by washing the organic phase with saturated aqueous sodium carbonate, 0.1M citric acid, and brine before drying with sodium sulfate. The solvents were then removed to yield crude product.

GENERAL PROCEDURE D

Fourth EDC Coupling Procedure

A round bottom flask was charged with the corresponding carboxylic acid (1.0 eq.), hydroxybenzotriazole hydrate (1.1 eq.) and the corresponding amine (1.0 eq.) in THF under nitrogen atmosphere. An appropriate amount (1.1 eq for free amines and 2.2 eq. for hydrochloride amine salts) of base, such as Hunig's base was added to the well stirred mixture followed by EDC (1.1 eq.). After stirring from 4 to 17 hours at room temperature the solvent was removed at reduced pressure, the residue taken up in ethyl acetate (or similar solvent) and water, washed with saturated aqueous sodium bicarbonate solution, 1 N HCl, brine, dried over anhydrous sodium sulfate and the solvent removed at reduced pressure to provide the product.

GENERAL PROCEDURE E

BOP Coupling Procedure

To a stirred solution of acid (2 mmol) in DMF, cooled in an ice-water bath, was added BOP (2.4 mmol) and N-methylmorpholine (6 mmol). The reaction mixture was stirred

for 50 min. and then a solution of amine (2 mmol) in DMF cooled at 0ºC was added. The cooling bath was allowed to warm to ambient temperature over 1-2 h and the reaction mixture was then stirred overnight. A 20% aqueous potassium carbonate solution (60 mL) was added and this mixture shaken thoroughly. No solid formed. The mixture was then washed with ethyl acetate (150 mL) and evaporated to dryness under vacuum to give a white solid. Water (50 mL) was then added and this mixture shaken thoroughly. The precipitate that formed was collected by filtration, then washed thoroughly with water, followed by 1 mL of diethyl ether to give the product.

GENERAL PROCEDURE F

Coupling of an Acid Chloride with an Amine

To a stirred solution of amine (4.6 mmol) in 5 ml of pyridine was added 4.6 mmol of the acid chloride. The mixture was stirred for 3.5 h to 48 h, dissolved in 100 mL of diethyl ether, washed with 10% HCl three times, brine once, 20% potassium carbonate once and brine once. The solution was dried over magnesium sulfate, filtered, and evaporated to yield the product. Other amino acid esters may also be employed in this procedure.

GENERAL PROCEDURE G

Coupling of an Acid with an Amine

A solution of the acid (3.3 mmol) and 1,1'-carbodiimidazole (CDI) in 20 mL THF was stirred for 2 h. amine hydrochloride (3.6 mmol) was added, followed by 1.5 mL (10.8 mmol) of triethylamine. The reaction mixture was stirred overnight. The reaction mixture was dissolved in 100 mL of diethyl ether, washed with 10% HCl three times, brine once, 20% potassium carbonate once and brine once. The solution was dried over magnesium sulfate, filtered, and evaporated to yield the product. Other amino acid esters may also be employed in this procedure.

GENERAL PROCEDURE H

Fifth EDC Coupling Procedure

In a round bottom flask was added an acid (1.1 eq.) in THF, an amine hydrochloride (1.0 eq.), 1-hydroxybenzotriazole hydrate (1.1 eq.), N,N-diisopropylethylamine (2.1 eq.), followed by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (1.1 eq.). The reaction mixture stirred at room temperature for 10-20 hours under an atmosphere of nitrogen. The mixture was diluted with EtOAc and washed with 0.1 M HCl (1 x 10 mL), saturated $NaHCO_3$ (1 x 10 mL), H_2O (1 x 10 mL), and brine and dried over $MgSO_4$. The drying agent was removed by filtration and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel followed by trituration from EtOAc and hexanes.

GENERAL PROCEDURE I

Sixth EDC Coupling Procedure

To a solution or suspension of the amine or amine hydrochloride (1.0 eq.) in THF (0.05-0.1 M) under N_2 at 0ºC was added the carboxylic acid (1.0-1.1 eq.), hydroxybenzotriazole monohydrate (1.1-1.15 eq.), Hunig's base (1.1 eq. for free amines and 1.1-2.3 eq. for hydrochloride amine salts), followed by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.1-1.15 eq.). The cooling bath was removed and the mixture allowed to warm to room temperature for 10-24 hours. The solution or mixture was diluted with EtOAc, in a 3-5 volume multiple of the initial THF volume, and washed with 0.1-1.0 M aq. HCl (1 or 2x), dilute $NaHCO_3$ (1 or 2x), and brine (1x). Then, the organic phase was dried over either $MgSO_4$ or Na_2SO_4, filtered, concentrated to provide the crude product, which was either further purified or utilized without further purification.

GENERAL PROCEDURE J

EEDQ Coupling Procedure

To a solution of the amine in THF (1.0 eq., 0.05-0.08 M, final molarity) under N_2 at room temperature was added the N-t-Boc protected β-amino acid (1.1 eq., either as a solid or in THF via cannula), followed by EEDQ (Aldrich, 1.1 eq.). The pale yellow solution was stirred at room temperature for 16-16.5 hours, then diluted with EtOAc (in a 3-5 volume multiple of the initial THF volume), and washed with 1M aq. HCl (2x), dilute aq. $NaHCO_3$ (2x), and brine (1x). The organic phase was dried over either Na_2SO_4 or $MgSO_4$, filtered, and concentrated.

GENERAL PROCEDURE K

Boc Protection of Amino Acids

The amine was stirred in 2 eq. of 1.0N sodium hydroxide at 0°C. 1.1 eq. of Boc-anhydride in dioxane was added dropwise via addition funnel. After addition was complete, the reaction was allowed to warm to room temperature, and stirring continued for a minimum of 17 hours. 5% $KHSO_4$ solution was added to acidify, and the mixture extracted with methylene chloride and ethyl acetate. The organics were combined, dried, and concentrated.

GENERAL PROCEDURE L

BOC Removal Procedure

A stream of anhydrous HCl gas was passed through a stirred solution of the N-t-Boc protected amine in 1,4-dioxane (0.03-0.09 M), chilled in a ice bath to ~10ºC under N_2, for 10-15 minutes. The solution was capped, the cooling bath removed, and the solution was allowed to warm to room temperature with stirring for 2-8 hours, monitoring by TLC for the consumption of starting material. The solution was concentrated (and in some instances dissolved in CH_2Cl_2 then re-concentrated and placed in vacuum oven at 60-70ºC to

remove most of the residual dioxane) and used without further purification.

GENERAL PROCEDURE M

Seventh EDC Coupling

To a 8 mL vial was added 150 μmol of a t-Boc protected β-amino acid and 1.5 mL of 10% DMF in methylene chloride. To this was added 0.8 mL (about 175 μmol) of a stock solution prepared by combining 5-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one hydrochloride (0.481 g, 1.75 mmol) and PP-HOBt (0.671 g, 1.75 mmol) in DMF (7.5 mL). Add 2.0 mL (about 200 μmol) of a stock solution prepared by combining EDC HCl (0.383 g, 2.0 mmol) in methylene chloride. After 14 hours of tumbling, the reaction mixture was combined with PS-piperidine resin (100-124 mg, about 3.6 mmol/g, Bruce, BG8-22P-177) and mixed for 15 minutes. Methanol (2.5 mL) was added to the vial and the contents applied to a pre-washed (methanol) 1000 mg SCX column using an 5 mL of methanol and then 5 mL of 10% methanol/chloroform. The coupled product is obtained by rinsing the column with 10% methanol/chloroform. The product containing fractions are combined and evaporated in vacuo and then dried at 40-45ºC under vacuum. The dried coupling product was dissolved in dioxane (5 mL), adding methanol, if needed, and evaporated in vacuo and again dried at 40-45ºC under vacuum.

The coupling product was then combined with hydrochloric acid in dioxane (5 mL, 4M, 20 mmol). After 2-3 hours the solvent was evaporated in vacuo and then dried at 40-45ºC under vacuum.

The deprotected coupling product was combined with 0.86 mL (150 μmol) of a stock solution of PP-HOBt prepared by combining PP-HOBt(0.567 g, 1.48 mmol) and 8.5 mL of. An additional 0.86 mL of DMF was added and the solution was partition into 4 vials. A 0.1 M stock solution of a carboxylic acid (about 40 μmol) in 10% DMF/methylene

chloride and then 0.4 mL (about 40 μmol) of a stock solution prepared by combining EDC HCl (0.383 g, 2.0 mmol) in methylene chloride (20 mL) were added to the vials. After 14 hours of tumbling, the reaction mixture was combined with PS-piperidine resin (100-124 mg, about 3.6 mmol/g, Bruce, BG8-22P-177) and mixed for 15 minutes. Methanol (2.5 mL) was added to the vial and the contents applied to a pre-washed (methanol) 1000 mg SCX column using an 5 mL of methanol and then 5 mL of 10% methanol/chloroform. The coupled product is obtained by rinsing the column with 10% methanol/chloroform. The product containing fractions are combined and evaporated in vacuo and then dried at 40-45°C under vacuum to give the product.

PREPARATION 1

Synthesis of (S)-5-Amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one Hydrochloride

A round bottom flask was charged with sodium hydride (0.295 g, 7.46 mmol) in 9.0 ml of DMF and treated with 5,7-dihydro-6H-dibenz(b,d)azepin-6-one (1.3 g, 6.22 mmol) (CAS # 20011-90-9, prepared as described in Brown, et. al., Tetrahedron Letters, No. 8, 667-670, (1971) and references cited therein). After stirring at 60°C for 1 h, the solution was treated with methyl iodide (1.16 ml, 18.6 mmol) and stirring continued for 17 h with the exclusion of light. After cooling, the reaction was diluted with CH_2Cl_2/H_2O, washed with $NaHSO_4$ solution, H_2O, and dried over Na_2SO_4. Evaporation and flash chromatography (SiO_2, $CHCl_3$) gave 0.885 g (63%) of 7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one as a colorless solid. $C_{15}H_{13}NO$ (MW = 223.27); mass spectroscopy (MH^+) 223. Anal. Calcd. for $C_{15}H_{13}NO$; C, 80.69 H, 5.87 N, 6.27. Found: C, 80.11 H, 5.95 N, 6.23. NMR data was as follows: ^{1}H-NMR ($CDCl_3$): δ = 7.62 (d, 2H), 7.26-7.47 (m, 6H), 3.51 (m, 2H), 3.32 (s, 3H).

The compound isolated above (0.700 g, 3.14 mmol) was dissolved in 20 ml of toluene and treated with butyl nitrite

(0.733 ml, 6.28 mmol). The reaction temperature was lowered to 0ºC and the solution was treated with KHMDS (9.42 ml, 0.5 M) under N_2 atmosphere. After stirring for 1 h the reaction was quenched with a saturated solution of $NaHSO_4$, diluted with CH_2Cl_2 and separated. The organic layer was dried over Na_2SO_4 and the title compound purified by chromatography (SiO_2, 98:2 $CHCl_3$/MeOH) giving 0.59 g (80 %) of 7-methyl-5-oximo-5,7-dihydro-6H-dibenz(b,d)azepin-6-one as a colorless solid. $C_{15}H_{12}N_2O_2$ (MW = 252.275); mass spectroscopy (MH^+) 252. Anal. Calcd. for $C_{15}H_{12}N_2O_2$; C, 71.42 H, 4.79 N, 11.10. Found: C, 71.24 H, 4.69 N, 10.87.

The oxime isolated above (0.99 g, 3.92 mmol) was hydrogenated in a Parr apparatus at 35 psi over 10 % Pd/C (0.46 g) in 3A ethanol. After 32 h the reaction mixture was filtered through a plug of Celite, the filtrate evaporated to a foam and treated with a saturated solution of HCl (g) in Et_2O. The resulting colorless solid was filtered, rinsed with cold Et_2O and vacuum dried to give 0.66 g (61 %) of 5-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one hydrochloride. $C_{15}H_{14}N_2O \cdot HCl$ (MW = 274.753); mass spectroscopy (MH+ free base) 238. Anal. Calcd. for $C_{15}H_{14}N_2O \cdot HCl$; C, 65.57 H, 5.50 N, 10.19 Found: C, 65.27 H, 5.67 N, 10.13. NMR data was as follows: ^{1}H-nmr ($DMSOd_6$): δ = 9.11 (bs, 3H), 7.78-7.41 (m, 8H), 4.83 (s, 1H), 3.25 (s, 3H).

Using racemic 5-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one (1.0 eq.) and di-p-toluoyl-D-tartaric acid monohydrate (1.0 eq.) in methanol, the title compound was prepared, after isolation of the base and formation of the hydrochloride as described above, as a solid. The product was collected by filtration. Enantiomeric excess was determined by chiral HPLC. Desired enantiomer 1: retention time of 9.97 minutes. Undesired enantiomer 2: retention time of 8.62 minutes. $C_{15}H_{15}ClN_2O$ (MW = 274.75); mass spectroscopy (MH^+) 239.1. Anal. Calcd. for $C_{15}H_{15}ClN_2O_3$; C, 65.57; H, 5.50; N, 10.20; Found: C, 65.51, H, 5.61; N, 10.01. NMR data was as

follows: ^{1}H-NMR (CDCl3): δ = 9.39 (s, 2H), 7.75-7.42 (m, 8H), 4.80 (s, 1H), 3.30 (s, 3H).

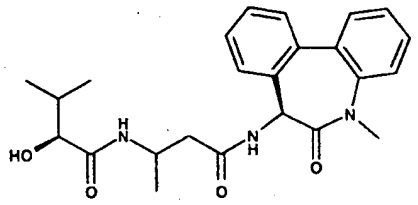
PREPARATION 2

Synthesis of (R/S)-N-Boc-β-methyl-β-alanine

Following General Procedure K and using D,L-3-aminobutyric acid (Aldrich), the title compound was prepared. $C_9H_{17}NO_4$ (MW=203.24); mass spectroscopy (MH+) 204. ^{1}H NMR data as follows: ^{1}H NMR (400 MHz, CD_3OD) δ 3.9 (1H q, J=3.9 Hz), δ 2.5 (1H dd, J=6.9, 16.1 Hz), δ 2.3 (1H dd, J=7.3, 15.2 Hz), δ 1.4 (9H s), δ 1.1 (3H d, J=6.8 Hz).

EXAMPLE 1

Synthesis of 5-(S)-N'-(N''-((S)-2-Hydroxy-3-methylbutyrl)-(R)-β-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one and 5-(S)-N'-(N''-((S)-2-Hydroxy-3-methylbutyrl)-(S)-β-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one



Following General Procedure D and using (R/S)-N-Boc-β-methyl-β-alanine and 5-(S)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one hydrochloride 5-(S)-N'-(N''-Boc-(R/S)-β-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one was prepared. $C_{24}H_{29}N_3O_4$ (MW=423.52); mass spectroscopy (MH^+) 424. ^{1}H NMR data as follows: ^{1}H NMR (400 MHz, CD_3OD) δ 8.54 (1H s), δ 7.62-7.35 (8H m), δ 5.24 (1H d, J=7.8 Hz), δ 3.98-3.91 (1H m), δ 3.27 (3H s), δ 2.60-2.38 (2H m), δ 1.35 (9H s), δ 1.12 (3H d, J=6.8 Hz).

Following General Procedure L using no cooling bath, and using 5-(S)-N'-((R/S)-N''-Boc-β-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one, 5-

(S)-N'-((R/S)-β-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one hydrochloride was prepared. $C_{19}H_{21}N_3O_2$ (MW=323.40); mass spectroscopy (MH+) 324. ^{1}H NMR data as follows: ^{1}H NMR (400 MHz, CD_3OD) δ 7.80-7.38 (8H m), δ 5.48 (1H s), δ 3.63-3.60 (1H m), δ 3.47 (3H s), δ 3.01-2.70 (2H m), δ 1.42 (3H d, J=6.5 Hz).

Following General Procedure D using (S)-2-hydroxy-3-methylbutyric acid (Aldrich) and 5-(S)-N'-((R/S)-β-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one hydrochloride, the title compounds were prepared. The isomers were separated via reverse phase HPLC, using Vydac C_{18} column (0.46 x 25cm), UV@214 nm, gradient of 5% to 70% (over 45 minutes) of 0.1% TFA/CH_3CN in 0.1% TFA/H_2O.

Isomer 1: $C_{24}H_{29}N_3O_4$ (MW=423.52); mass spectroscopy (MH+) 424. ^{1}H NMR data as follows: ^{1}H NMR (400 MHz, CD_3OD) δ 7.63-7.35 (8H m), δ 5.27 (1H s), δ 4.31-4.26 (1H m), δ 3.73 (1H d, J=3.9 Hz), δ 3.72 (3H s), δ 2.69 (1H dd, J=6.3, 14.1 Hz), δ 2.52 (1H dd, J=6.4, 13.8 Hz), δ 1.99-1.94 (1H m), δ 1.18 (3H d, J=6.8 Hz), δ 0.89 (3H d, J=6.8 Hz), δ 0.70 (3H d, 6.8 Hz).

Isomer 2: $C_{24}H_{29}N_3O_4$ (MW=423.52); mass spectroscopy (MH+) 424. ^{1}H NMR data as follows: ^{1}H NMR (400 MHz, CD_3OD) δ 7.64-7.33 (8H m), δ 5.28 (1H s), δ 4.33-4.30 (1H m), δ 3.73 (1H d, J=3.4 Hz), δ 3.28 (3H s), δ 2.64 (1H dd, J=8.1, 13.9), δ 2.54 (1H dd, J=5.6, 13.9), δ 2.02-1.96 (1H m), δ 1.18 (3H d, J=6.4), δ 0.94 (3H d, J=6.5 Hz), δ 0.88 (3H d, J=6.4 Hz).

PREPARATION 3

Synthesis of S-(+)-3,5-Difluoromandelic Acid

To a solution of 3,5-difluorobenzaldehyde (Aldrich) in CH_2Cl_2 (100 mL) was added $ZnCl_2$ (6.7 g, 21.1 mmol) to form a slurry. Trimethysilyl cyanide (21.0 g, 211.2 mmol) dissolved in CH_2Cl_2 (100 mL) was slowly added to the slurry at 0ºC. The resulting solution was stirred at room temperature for 4 h. The reaction mixture was then diluted with water and the organic layer separated. The combined

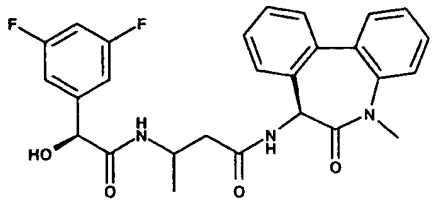
organic layers were concentrated to a residue. The residue was dissolved with MeOH (200 mL) at 0ºC and anhydrous HCl gas was bubbled into the solution for 10 min. After stirring at room temperature for 18 h, the solution was concentrated to a solid. The solid was dissolved in CH_2Cl_2/H_2O and the aqueous portion extracted with CH_2Cl_2. The combined organics were washed with brine, dried over anhydrous $MgSO_4$ and concentrated to give methyl (±)-3,5-difluoromandelate as a solid (37.4 g, 87.6%), mp = 77-78ºC. ^1H NMR (300 MHz, $CDCl_3$): δ = 6.97 (dd, J = 9.6 Hz, J = 1.79 Hz, 2H), 6.74 (dt, J = 8.82, J = 2.28 Hz, 1H), 5.14 (d, J = 4.64 Hz, 1H), 3.78 (s, 3H), 3.54 (d, J = 5.1 Hz, 1H).

Methyl (±)-3,5-difluoromandelate was separated via preparative chiral HPLC to give methyl S-(+)-3,5-difluoromandelate as a white solid having a melting point of 70-71ºC. $C_9H_8F_2O_3$ (MW = 202.17); mass spectroscopy found (M+NH4+) 220.0. Anal. Calcd. for $C_9H_8F_2O_3$: C, 53.47; H, 3.99. Found: C, 53.40; H, 3.89.

A solution of methyl S-(+)-3,5-difluoromandelate (1 eq.) in 74% aqueous THF was cooled to 0ºC and treated with lithium hydroxide. After 40 minutes at 0ºC the reaction was complete by TLC. The contents were transferred to a separatory funnel and partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. The aqueous layer was acidified with 0.5 N $NaHSO_4$ and extracted thrice with ethyl acetate. The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated to a white solid having a melting point of 119-122ºC. The ^1H NMR was consistent with known 3,5-difluoromandelic acid.

EXAMPLE 2

Synthesis of 5-(S)-N'-(N''-((S)-3,5-difluorophenyl-α-hydroxyacetyl)-(R)-β-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one and 5-(S)-N'-(N''-((S)-3,5-difluorophenyl-α-hydroxyacetyl)-(S)-β-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one



Following General Procedure D using (S)-3,5-difluoromandelic acid and 5-(S)-N'-((R/S)-β-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one hydrochloride the title compounds were prepared.

Isomer 1: $C_{27}H_{25}F_2N_3O_4$ (MW=493.51); mass spectroscopy (MH+) 494. Anal. Calcd. for $C_{27}H_{25}F_2N_3O_4$: C 65.71, H 6.16, N 8.51; Found: C 65.02, H 6.16, N 7.64.

Isomer 2: $C_{27}H_{25}F_2N_3O_4$ (MW=493.51); mass spectroscopy (MH+) 494. Anal. Calcd. for $C_{27}H_{25}F_2N_3O_4$: C 65.71, H 6.16, N 8.51; Found: C 64.59, H 5.33, N 7.98.

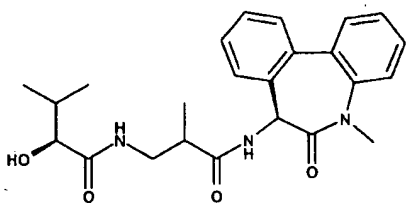
PREPARATRION 4

Synthesis of (R/S)-N-Boc-α-methyl-β-alanine.

Following General Method K and using D,L-3-aminoisobutyric acid (Aldrich), the title compound was prepared. $C_9H_{17}NO_4$ (MW=203.24); mass spectroscopy (MH+) 204. ^1H NMR data as follows: ^1H NMR (400 MHz, CD_3OD) δ 3.21 (1H dd, J=7.3, 13.6 Hz), δ 3.01 (1H dd, J=6.3, 13.1 Hz), δ 2.55 (1H q, J=6.8 Hz), δ 1.36 (9H s), δ 1.08 (3H d, J=7.3 Hz).

EXAMPLE 3

Synthesis of 5-(S)-N'-(N''-((S)-2-Hydroxy-3-methylbutyrl)-(R)-α-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one and 5-(S)-N'-(N''-((S)-2-Hydroxy-3-methylbutyrl)-(S)-α-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one



Following General Procedure D and using (R/S)-N-Boc-β-methyl-β-alanine and 5-(S)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one hydrochloride, 5-(S)-N'-(N''-Boc-(R/S)-α-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one was prepared. $C_{24}H_{29}N_3O_4$ (MW=423.52); mass spectroscopy (MH+) 424. ^{1}H NMR data as follows: ^{1}H NMR (400 MHz, CD_3OD) δ 7.64-7.33 (8H m), δ 5.26 (1/2H s), δ 5.25 (1/2H s) δ 3.28 (3H s), δ 3.20-3.04 (2H m), δ 2.96-2.80 (1H m), δ 1.42 (4.5H s), δ 1.39 (4.5H s), δ 1.16 (1.5H d, J=6.8 Hz), δ 1.10 (1.5H d, J=6.7 Hz).

Following General Procedure L, using no cooling bath, and using 5-(S)-((R/S)-N'-Boc-β-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one, 5-(S)-N'-((R/S)-α-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one hydrochloride was prepared. $C_{19}H_{21}N_3O_2$ (MW=323.40); mass spectroscopy (MH^+) 324. ^{1}H NMR data as follows: ^{1}H NMR (400 MHz, CD_3OD) δ 7.65-7.37 (8H m), δ 5.34 (1/2H s), δ 5.32 (1/2H s), δ 3.29 (3H s), δ 3.19-2.96 (3H m), δ 1.31 (3H d, J=6.4Hz).

Following General Procedure D using (S)-2-hydroxy-3-methylbutyric acid (Aldrich) and 5-(S)-N'-((R/S)-α-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one hydrochloride the title compounds were prepared. The isomers were partially separated via reverse phase HPLC,

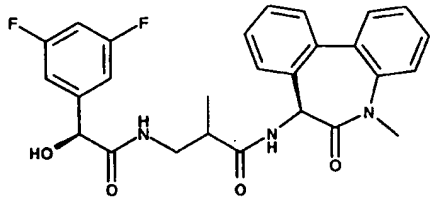
using Vydac C18 column (0.46 x 25cm), UV@214 nm, gradient of 5% to 70% (over 45 minutes) of 0.1% TFA/CH_3CN in 0.1% TFA/H_2O.

Isomer 1: (62.5% enriched) $C_{24}H_{29}N_3O_4$ (MW=423.52); mass spectroscopy (MH+) 424. ^1H NMR data as follows: ^1H NMR (400 MHz, CD3OD) δ 7.62-7.37 (8H m), δ 5.26 (1H s), δ 3.80 (1H d, J=3.9 Hz), δ 3.42-3.29 (2H m), δ 3.27 (3H s), δ 2.93 (1H q, J=6.3 Hz), δ 2.04-1.96 (1H m), δ 1.12 (3H d, J=6.8 Hz), δ 0.92 (3H d, J=6.8 Hz), δ 0.75 (3H d, J=6.8 Hz).

Isomer 2: (58.3% enriched) $C_{24}H_{29}N_3O_4$ (MW=423.52); mass spectroscopy (MH+) 424. ^1H NMR data as follows: ^1H NMR (400 MHz, CD_3OD) δ 7.63-7.32 (8H m), δ 5.25 (1H s), δ 3.75 (1H d, J=3.9 Hz), δ 3.36-3.31 (2H m), δ 3.27 (3H s), δ 2.95-2.91 (1H m), δ 2.01-1.97 (1H m), δ 1.18 (3H d, J=6.8 Hz), δ 0.93 (3H d, J=6.7 Hz), δ 0.74 (3H d, J=6.8 Hz).

EXAMPLE 4

Synthesis of 5-(S)-N'-(N''-((S)-3,5-difluorophenyl-α-hydroxyacetyl)-(R)-α-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one and 5-(S)-N'-(N''-((S)-3,5-difluorophenyl-α-hydroxyacetyl)-(S)-α-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one



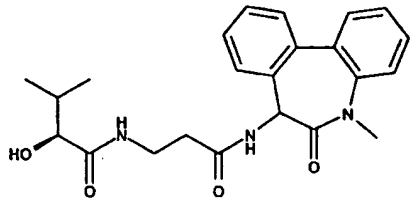
Following General Procedure D using (S)-3,5-difluoromandelic acid and 5-(S)-N'-((R/S)-α-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one hydrochloride, the title compounds were prepared. The isomers were partially separated via reverse phase HPLC, using Vydac C18 column (0.46 x 25cm), UV@214 nm, gradient of 5% to 70% (over 45 minutes) of 0.1% TFA/CH_3CN in 0.1% TFA/H_2O.

Isomer 1: $C_{27}H_{25}F_2N_3O_4$ (MW=493.51); mass spectroscopy (MH^+) 494. ^1H NMR data as follows: ^1H NMR (400 MHz, CD_3OD) δ 7.60-7.32 (8H m), δ 6.95 (2H d, J=5.9 Hz), δ 6.72-6.70 (1H m), δ 5.20 (1H s), δ 4.95 (1H s), δ 3.34-3.18 (5H m), δ 2.89-2.86 (1H m), δ 1.04 (3H d, J=6.8 Hz).

Isomer 2: (78.3% enriched) $C_{27}H_{25}F_2N_3O_4$ (MW=493.51); mass spectroscopy (MH^+) 494. ^1H NMR data as follows: ^1H NMR (400 MHz, CD_3OD) δ 7.59-7.25 (8H m), δ 6.97-6.94 (2H m), δ 6.75-6.72 (1H m), δ 5.18 (1H s), δ 4.90 (1H s), δ 3.28-3.16 (5H m), δ 2.88-2.80 (1H m), 1.08 (3H d, J=6.8 Hz).

EXAMPLE 5

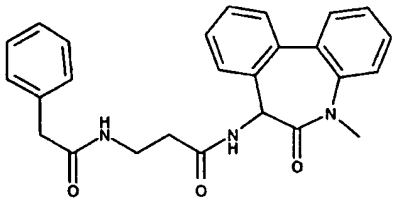
Synthesis of 5-(R)-N'-(N''-((S)-2-Hydroxy-3-methylbutyrl)-(β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one and 5-(S)-N'-(N''-((S)-2-Hydroxy-3-methylbutyrl)-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one



Following General Procedure M and using N-Boc-β-alanine and 5-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one hydrochloride in the first coupling and (S)-hydroxyvaleric acid in the second coupling gave the title compound.

EXAMPLE 6

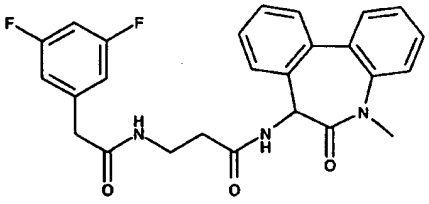
Synthesis of 5-(R)-N'-(N''-(phenylacetyl)-(β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one and 5-(S)-N'-(N''-(phenylacetyl)-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one



Following General Procedure M and using N-Boc-β-alanine and 5-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one hydrochloride in the first coupling and phenylacetic acid in the second coupling gave the title compound.

EXAMPLE 7

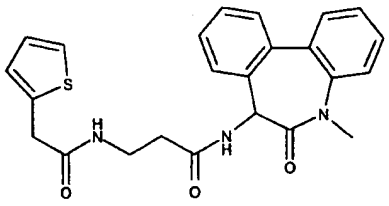
Synthesis of 5-(R)-N'-(N''-(3,5-difluorophenylacetyl)-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one and 5-(S)-N'-(N''-(3,5-difluorophenylacetyl)-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one



Following General Procedure M and using N-Boc-β-alanine and 5-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one hydrochloride in the first coupling and 3,5-difluorophenylacetic acid in the second coupling gave the title compound.

EXAMPLE 8

Synthesis of 5-(R)-N'-(N''-(thien-2-ylacetyl)-(β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one and 5-(S)-N'-(N''-(thien-2-ylacetyl)-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one



Following General Procedure M and using N-Boc-β-alanine and 5-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one hydrochloride in the first coupling and thien-2-ylacetic acid in the second coupling gave the title compound.

Example Bio-1

Cellular Screen for the Detection of Inhibitors of β-Amyloid Production

Numerous compounds of formula I above were assayed for their ability to inhibit β-amyloid production in a cell line possessing the Swedish mutation. This screening assay employed cells (K293 = human kidney cell line) which were stably transfected with the gene for amyloid precursor protein 751 (APP751) containing the double mutation Lys651Met652 to Asn651Leu652 (APP751 numbering) in the manner described in International Patent Application Publication No. 94/105698 and Citron, et al., Nature, 360:672-674 (1992). This mutation is commonly called the Swedish mutation and the cells, designated as "293 751 SWE", were plated in Corning 96-well plates at 2-4 x 104 cells per well in Dulbecco's minimal essential media (Sigma, St. Louis, MO) plus 10% fetal bovine serum. Cell number is important in order to achieve β-amyloid ELISA results within the linear range of the assay (~0.2 to 2.5 ng per mL).

Following overnight incubation at 37ºC in an incubator equilibrated with 10% carbon dioxide, media were removed and replaced with 200 μL of a compound of formula I (drug) containing media per well for a two hour pretreatment period and cells were incubated as above. Drug stocks were prepared in 100% dimethyl sulfoxide such that at the final drug concentration used in the treatment, the concentration of dimethyl sulfoxide did not exceed 0.5% and, in fact, usually equaled 0.1%.

At the end of the pretreatment period, the media were again removed and replaced with fresh drug containing media as above and cells were incubated for an additional two hours. After treatment, plates were centrifuged in a Beckman GPR at 1200 rpm for five minutes at room temperature to pellet cellular debris from the conditioned media. From each well, 100 μL of conditioned media or appropriate dilutions thereof were transferred into an ELISA plate precoated with antibody 266 (P. Seubert, Nature (1992) 359:325-327) against amino acids 13-28 of β-amyloid peptide as described in International Patent Application Publication No. 94/105698 and stored at 4ºC overnight. An ELISA assay employing labeled antibody 3D6 (P. Seubert, Nature (1992) 359:325-327) against amino acids 1-5 of β-amyloid peptide was run the next day to measure the amount of β-amyloid peptide produced.

Cytotoxic effects of the compounds were measured by a modification of the method of Hansen, et al.13. To the cells remaining in the tissue culture plate was added 25 μL of a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO) stock solution (5 mg/mL) to a final concentration of 1 mg/mL. Cells were incubated at 37ºC for one hour, and cellular activity was stopped by the addition of an equal volume of MTT lysis buffer (20% w/v sodium dodecylsulfate in 50% dimethylformamide, pH 4.7). Complete extraction was achieved by overnight shaking at room temperature. The

difference in the OD_{562nm} and the OD_{650nm} was measured in a Molecular Device's UV_{max} microplate reader as an indicator of the cellular viability.

The results of the β-amyloid peptide ELISA were fit to a standard curve and expressed as ng/mL β-amyloid peptide. In order to normalize for cytotoxicity, these results were divided by the MTT results and expressed as a percentage of the results from a drug free control. The test compounds were assayed for β-amyloid peptide production inhibition activity in cells using this assay.

Example Bio-2

In Vivo Suppression of β-Amyloid Release and/or Synthesis

This example illustrates how the compounds of this invention could be tested for in vivo suppression of β-amyloid release and/or synthesis. For these experiments, 3 to 4 month old PDAPP mice are used (Games et al., (1995) Nature 373:523-527). Depending upon which compound is being tested, the compound is usually formulated at between 1 and 10 mg/mL. Because of the low solubility factors of the compounds, they may be formulated with various vehicles, such as corn oil (Safeway, South San Francisco, CA); 10% ethanol in corn oil; 2-hydroxypropyl-β-cyclodextrin (Research Biochemicals International, Natick MA); and carboxy-methyl-cellulose (Sigma Chemical Co., St. Louis MO).

The mice are dosed subcutaneously with a 26 gauge needle and 3 hours later the animals are euthanized via CO_2 narcosis and blood is taken by cardiac puncture using a 1 cc 25G 5/8" tuberculin syringe/needle coated with solution of 0.5 M EDTA, pH 8.0. The blood is placed in a Becton-Dickinson vacutainer tube containing EDTA and spun down for 15 minutes at 1500 xg at 5ºC. The brains of the mice are then removed and the cortex and hippocampus are dissected out and placed on ice.

1. Brain Assay

To prepare hippocampal and cortical tissue for enzyme-linked immunosorbent assays (ELISAs) each brain region is homogenized in 10 volumes of ice cold guanidine buffer (5.0 M guanidine-HCl, 50 mM Tris-HCl, pH 8.0) using a Kontes motorized pestle (Fisher, Pittsburgh PA). The homogenates are gently rocked on a rotating platform for three to four hours at room temperature and stored at -20ºC prior to quantitation of β-amyloid.

The brain homogenates are diluted 1:10 with ice-cold casein buffer (0.25% casein, phosphate buffered saline (PBS), 0.05% sodium azide, 20 µg/ml aprotinin, 5 mM EDTA, pH 8.0, 10 µg/ml leupeptin), thereby reducing the final concentration of guanidine to 0.5 M, before centrifugation at 16,000 xg for 20 minutes at 4ºC. Samples are further diluted, if necessary, to achieve an optimal range for the ELISA measurements by the addition of casein buffer with 0.5 M guanidine hydrochloride added. The β-amyloid standards (1-40 or 1-42 amino acids) were prepared such that the final composition equaled 0.5 M guanidine in the presence of 0.1% bovine serum albumin (BSA).

The total β-amyloid sandwich ELISA, quantitating both β-amyloid (aa 1-40) and β-amyloid (aa 1-42) consists of two monoclonal antibodies (mAb) to β-amyloid. The capture antibody, 266 (P. Seubert, Nature (1992) 359:325-327), is specific to amino acids 13 - 28 of β-amyloid. The antibody 3D6 (Johnson-Wood et al., PNAS USA (1997) 94:1550-1555), which is specific to amino acids 1 - 5 of β-amyloid, is biotinylated and served as the reporter antibody in the assay. The 3D6 biotinylation procedure employs the manufacturer's (Pierce, Rockford IL) protocol for NHS-biotin labeling of immunoglobulins except that 100 mM sodium bicarbonate, pH 8.5 buffer is used. The 3D6 antibody does not recognize secreted amyloid precursor protein (APP) or full-length APP but detects only β-amyloid species with an amino terminal aspartic acid. The assay has a lower limit

of sensitivity of ~50 pg/ml (11 pM) and shows no cross-reactivity to the endogenous murine β-amyloid peptide at concentrations up to 1 ng/ml.

The configuration of the sandwich ELISA quantitating the level of β-amyloid (aa 1-42) employs the mAb 21F12 (Johnson-Wood et al., PNAS USA (1997) 94:1550-1555) (which recognizes amino acids 33-42 of β-amyloid) as the capture antibody. Biotinylated 3D6 is also the reporter antibody in this assay which has a lower limit of sensitivity of ~125 pg/ml (28 pM).

The 266 and 21F12 capture mAbs are coated at 10 µg/ml into 96 well immunoassay plates (Costar, Cambridge MA) overnight at room temperature. The plates are then aspirated and blocked with 0.25% human serum albumin in PBS buffer for at least 1 hour at room temperature, then stored desiccated at 4ºC until use. The plates are dehydrated with wash buffer (Tris-buffered saline, 0.05% Tween 20) prior to use. The samples and standards are added to the plates and incubated overnight at 4ºC. The plates are washed • 3 times with wash buffer between each step of the assay. The biotinylated 3D6, diluted to 0.5 µg/ml in casein incubation buffer (0.25% casein, PBS, 0.05% Tween 20, pH 7.4) is incubated in the well for 1 hour at room temperature. Avidin-HRP (Vector, Burlingame CA) diluted 1:4000 in casein incubation buffer is added to the wells for 1 hour at room temperature. The colorimetric substrate, Slow TMB-ELISA (Pierce, Cambridge MA), is added and allowed to react for 15 minutes, after which the enzymatic reaction is stopped with addition of 2 N H_2SO_4. Reaction product is quantified using a Molecular Devices Vmax (Molecular Devices, Menlo Park CA) measuring the difference in absorbance at 450 nm and 650 nm.

2. Blood Assay

The EDTA plasma is diluted 1:1 in specimen diluent (0.2 gm/l sodium phosphate·H_2O (monobasic), 2.16 gm/l sodium phosphate·7 H_2O (dibasic), 0.5gm/l thimerosal, 8.5 gm/l

sodium chloride, 0.5 ml Triton X-405, 6.0 g/l globulin-free bovine serum albumin; and water). The samples and standards in specimen diluent are assayed using the total β-amyloid assay (266 capture/3D6 reporter) described above for the brain assay except the specimen diluent was used instead of the casein diluents described.

Formulations other than those described above can also be used for oral delivery and intravenous delivery to a mammal. For oral delivery, the compound can be mixed with either 100% corn oil or, alternatively, in a solution containing 80% corn oil, 19.5% oleic acid and 0.5% labrafil. The compound can be mixed with the above solutions in concentrations ranging from 1 mg/mL to 10 mg/mL. The compound in solution is preferably administered orally to the mammal at a dose volume of 5 mL/kg of body weight. For IV delivery, the compound is preferably mixed with a solution of 3% ethanol, 3% solutol HS-15 and 94% saline. The compound is preferably mixed with the above solution in concentrations ranging from 0.25 mg/mL to 5 mg/mL. The compound in solution is preferably administered by IV to the mammal at a dose volume of 2 mL/kg of body weight.

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When employed as pharmaceuticals, the compounds of formula I are usually administered in the form of pharmaceutical compositions. These compounds can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. These compounds are effective as both injectable and oral compositions. Such compositions are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound.

This invention also includes pharmaceutical compositions which contain, as the active ingredient, one or more of the compounds of formula I above associated with pharmaceutically acceptable carriers. In making the compositions of this invention, the active ingredient is usually mixed with an excipient, diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule, sachet, paper or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

In preparing a formulation, it may be necessary to mill the active

compound to provide the appropriate particle size prior to combining with the other ingredients. If the active compound is substantially insoluble, it ordinarily is milled to a particle size of less than 200 mesh. If the active compound is substantially water soluble, the particle size is normally adjusted by milling to provide a substantially uniform distribution in the formulation, e.g. about 40 mesh.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, sterile water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 5 to about 100 mg, more usually about 10 to about 30 mg, of the active ingredient. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient. Preferably, the compound of formula I above is employed at no more than about 20 weight percent of the pharmaceutical composition, more preferably no more than about 15 weight percent, with the balance being pharmaceutically inert carrier(s).

The active compound is effective over a wide dosage range and is generally administered in a pharmaceutically effective amount. It, will be understood, however, that the amount of the compound actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the

individual patient, the severity of the patient's symptoms, and the like.

For preparing solid compositions such as tablets, the principal active ingredient is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation is then subdivided into unit dosage forms of the type described above containing from, for example, 0.1 to about 500 mg of the active ingredient of the present invention.

The tablets or pills of the present invention may be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can separated by enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

The liquid forms in which the novel compositions of the present invention may be incorporated for administration orally or by injection include aqueous solutions suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described supra. Preferably the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutically acceptable solvents may be nebulized by use of inert gases. Nebulized solutions may be breathed directly from the nebulizing device or the nebulizing device may be attached to a face masks tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

The following formulation examples illustrate the pharmaceutical compositions of the present invention.

Formulation Example 1

Hard gelatin capsules containing the following ingredients are prepared:

Ingredient	Quantity (mg/capsule)
Active Ingredient	30.0
Starch	305.0
Magnesium stearate	5.0

The above ingredients are mixed and filled into hard gelatin capsules in 340 mg quantities.

Formulation Example 2

A tablet formula is prepared using the ingredients below:

Ingredient	Quantity (mg/tablet)
Active Ingredient	25.0
Cellulose, microcrystalline	200.0
Colloidal silicon dioxide	10.0
Stearic acid	5.0

The components are blended and compressed to form tablets, each weighing 240 mg.

Formulation Example 3

A dry powder inhaler formulation is prepared containing the following components:

Ingredient	Weight %
Active Ingredient	5
Lactose	95

The active ingredient is mixed with the lactose and the mixture is added to a dry powder inhaling appliance.

Formulation Example 4

Tablets, each containing 30 mg of active ingredient, are prepared as follows:

Ingredient	Quantity (mg/tablet)
Active Ingredient	30.0 mg
Starch	45.0 mg
Microcrystalline cellulose	35.0 mg
Polyvinylpyrrolidone (as 10% solution in sterile water)	4.0 mg
Sodium carboxymethyl starch	4.5 mg
Magnesium stearate	0.5 mg
Talc	1.0 mg
Total	120 mg

The active ingredient, starch and cellulose are passed through a No. 20 mesh U.S. sieve and mixed thoroughly. The solution of polyvinyl-pyrrolidone is mixed with the resultant powders, which are then passed through a 16 mesh U.S. sieve. The granules so produced are dried at 50º to 60ºC and passed through a 16 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 30 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 150 mg.

Formulation Example 5

Capsules, each containing 40 mg of medicament are made as follows:

Ingredient	Quantity (mg/capsule)
Active Ingredient	40.0 mg
Starch	109.0 mg
Magnesium stearate	1.0 mg
Total	150.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 150 mg quantities.

Formulation Example 6

Suppositories, each containing 25 mg of active ingredient are made as follows:

Ingredient	Amount
Active Ingredient	25 mg
Saturated fatty acid glycerides to	2,000 mg

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2.0 g capacity and allowed to cool.

Formulation Example 7

Suspensions, each containing 50 mg of medicament per 5.0 mL dose are made as follows:

Ingredient	Amount
Active Ingredient	50.0 mg
Xanthan gum	4.0 mg
Sodium carboxymethyl cellulose (11%)	
Microcrystalline cellulose (89%)	50.0 mg
Sucrose	1.75 g
Sodium benzoate	10.0 mg
Flavor and Color	q.v.
Purified water to	5.0 mL

The active ingredient, sucrose and xanthan gum are blended, passed through a No. 10 mesh U.S. sieve, and then mixed with a previously made solution of the microcrystalline cellulose and sodium carboxymethyl cellulose in water. The sodium benzoate, flavor, and color are diluted with some of the water and added with stirring. Sufficient water is then added to produce the required volume.

Formulation Example 8

Ingredient	Quantity (mg/capsule)
Active Ingredient	15.0 mg
Starch	407.0 mg
Magnesium stearate	3.0 mg
Total	425.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 560 mg quantities.

Formulation Example 9

A subcutaneous formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient	1.0 mg
corn oil	1 mL

(Depending on the solubility of the active ingredient in corn oil, up to about 5.0 mg or more of the active ingredient may be employed in this formulation, if desired).

Formulation Example 10

A topical formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient	1-10 g
Emulsifying Wax	30 g
Liquid Paraffin	20 g
White Soft Paraffin	to 100 g

The white soft paraffin is heated until molten. The liquid paraffin and emulsifying wax are incorporated and stirred until dissolved. The active ingredient is added and stirring is continued until dispersed. The mixture is then cooled until solid.

Another preferred formulation employed in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the compounds of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. See, e.g., U.S. Patent 5,023,252, issued June 11, 1991, herein incorporated by reference. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

Frequently, it will be desirable or necessary to introduce the pharmaceutical composition to the brain, either directly or indirectly. Direct techniques usually

involve placement of a drug delivery catheter into the host's ventricular system to bypass the blood-brain barrier. One such implantable delivery system used for the transport of biological factors to specific anatomical regions of the body is described in U.S. Patent 5,011,472 which is herein incorporated by reference.

Indirect techniques, which are generally preferred, usually involve formulating the compositions to provide for drug latentiation by the conversion of hydrophilic drugs into lipid-soluble drugs. Latentiation is generally achieved through blocking of the hydroxy, carbonyl, sulfate, and primary amine groups present on the drug to render the drug more lipid soluble and amenable to transportation across the blood-brain barrier. Alternatively, the delivery of hydrophilic drugs may be enhanced by intra-arterial infusion of hypertonic solutions which can transiently open the blood-brain barrier.

Other suitable formulations for use in the present invention can be found in Remington's Pharmaceutical Sciences, Mace Publishing Company, Philadelphia, PA, 17th ed. (1985).

The compounds and pharmaceutical compositions of the invention are useful in inhibiting β-amyloid peptide release and/or its synthesis, and, accordingly, have utility in diagnosing and treating Alzheimer's disease in mammals including humans.

As noted above, the compounds described herein are suitable for use in a variety of drug delivery systems described above. Additionally, in order to enhance the in vivo serum half-life of the administered compound, the compounds may be encapsulated, introduced into the lumen of liposomes, prepared as a colloid, or other conventional techniques may be employed which provide an extended serum half-life of the compounds. A variety of methods are available for preparing liposomes, as described in, e.g.,

Szoka, et al., U.S. Patent Nos. 4,235,871, 4,501,728 and 4,837,028 each of which is incorporated herein by reference.

The amount of compound administered to the patient will vary depending upon what is being administered, the purpose of the administration, such as prophylaxis or therapy, the state of the patient, the manner of administration, and the like. In therapeutic applications, compositions are administered to a patient already suffering from Alzheimer's disease in an amount sufficient to at least partially arrest further onset of the symptoms of the disease and its complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on the judgment of the attending clinician depending upon factors such as the degree or severity of Alzheimer's disease in the patient, the age, weight and general condition of the patient, and the like. Preferably, for use as therapeutics, the compounds described herein are administered at dosages ranging from about 1 to about 500 mg/kg/day.

In prophylactic applications, compositions are administered to a patient at risk of developing Alzheimer's disease (determined for example by genetic screening or familial trait) in an amount sufficient to inhibit the onset of symptoms of the disease. An amount adequate to accomplish this is defined as "prophylactically effective dose." Amounts effective for this use will depend on the judgment of the attending clinician depending upon factors such as the age, weight and general condition of the patient, and the like. Preferably, for use as prophylactics, the compounds described herein are administered at dosages ranging from about 1 to about 500 mg/kg/day.

As noted above, the compounds administered to a patient are in the form of pharmaceutical compositions described above. These compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. The

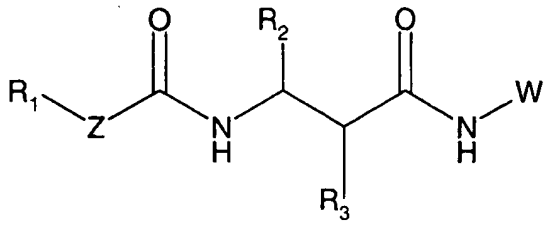
resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous carrier prior to administration. The pH of the compound preparations typically will be between 3 and 11, more preferably from 5 to 9 and most preferably from 7 and 8. It will be understood that use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of pharmaceutical salts.

The compounds described herein are also suitable for use in the administration of the compounds to a cell for diagnostic and drug discovery purposes. Specifically, the compounds may be used in the diagnosis of cells releasing and/or synthesizing β-amyloid peptide. In addition the compounds described herein are useful for the measurement and evaluation of the activity of other candidate drugs on the inhibition of the cellular release and/or synthesis of β-amyloid peptide.

From the foregoing description, various modifications and changes in the composition and method will occur to those skilled in the art. All such modifications coming within the scope of the appended claims are intended to be included therein.

WHAT IS CLAIMED IS:

1. A compound of the formula



formula I

wherein

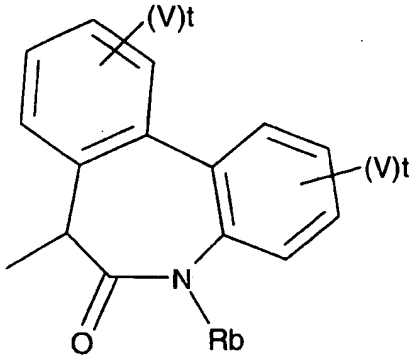
R_1 is selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, aryl, heteroaryl and heterocyclic;

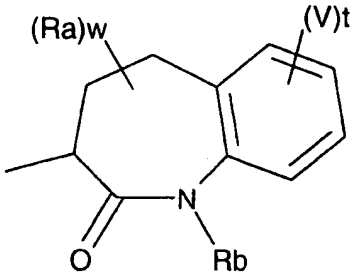
R_2 is selected from the group consisting of hydrogen, alkyl, cycloalkyl, and aryl;

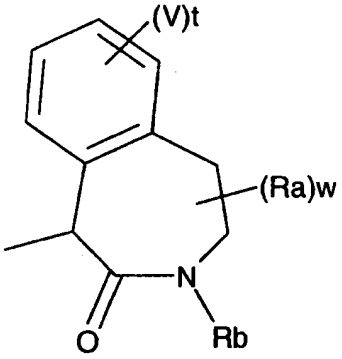
R_3 is selected from the group consisting of hydrogen, alkyl, cycloalkyl, and aryl;

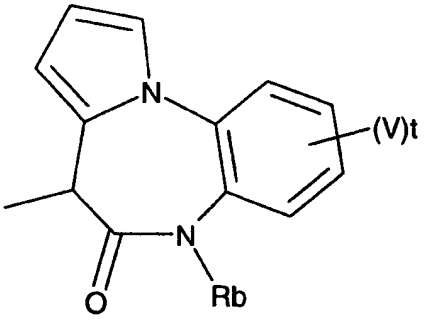
Z is represented by the formula -CX'X"-
wherein
X' is selected from the group consisting of hydrogen, hydroxy, and fluoro,
X" is selected from the group consisting of hydrogen, hydroxy, and fluoro, or
X' and X" together form an oxo group;

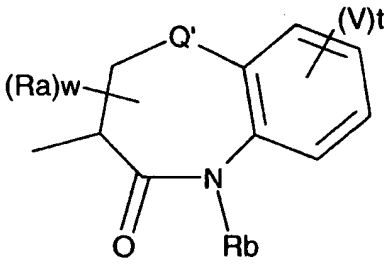
W is a cyclic group selected from the group consisting of

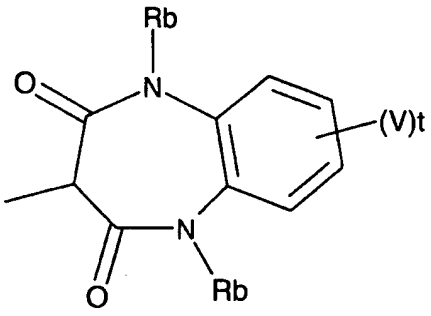


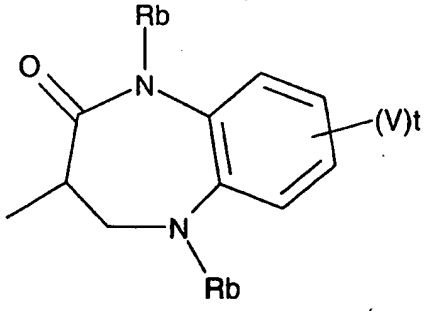


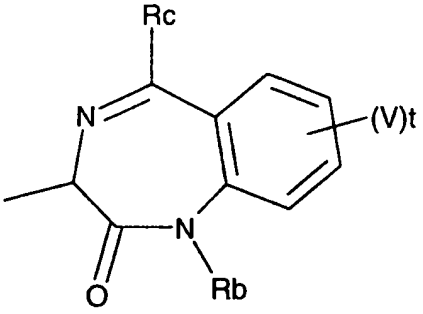












wherein
Q' is oxygen or sulfur;

each V is independently selected from the group consisting of hydroxy, acyl, acyloxy, alkyl, substituted alkyl, alkoxy, substituted alkoxy, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, amino, aminoacyl, alkaryl, aryl, aryloxy, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, thioalkoxy, substituted thioalkoxy, trihalomethyl;

Ra is selected from the group consisting of alkyl, substituted alkyl, alkoxy, substituted alkoxy, amino, carboxyl, carboxyl alkyl, cyano, halo;

Rb is selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, acyl, aryl, heteroaryl, heterocyclic;

Rc is selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, heteroaryl, heterocyclic, cycloalkyl, and substituted cycloalkyl;

t is an integer from 0 to 4;

w is an integer from 0 to 4;

and the pharmaceutically acceptable salts thereof.

2. A compound according to Claim 1 wherein R_1 is alkyl or aryl.

3. A compound according to Claim 2 wherein R_1 is C_1-C_4 alkyl.

4. A compound according to Claim 3 wherein the C_1-C_4 alkyl is isopropyl.

5. A compound according to Claim 1 wherein R_1 is phenyl substituted with from 1 to 3 substituents selected from the group consisting of hydrogen, alkyl, alkoxy, and halo.

6. A compound according to Claim 5 wherein R_1 is 3,5-difluorophenyl.

7. A compound according to Claim 1 wherein one of R_2 or R_3 is hydrogen.

8. A compound according to Claim 7 wherein the one of R_2 or R_3 is alkyl or aryl.

9. A compound according to Claim 8 wherein the alkyl is C_1-C_4 alkyl.

10. A compound according to Claim 9 wherein the C_1-C_4 alkyl is methyl.

11. A compound according to Claim 8 wherein the aryl is phenyl.

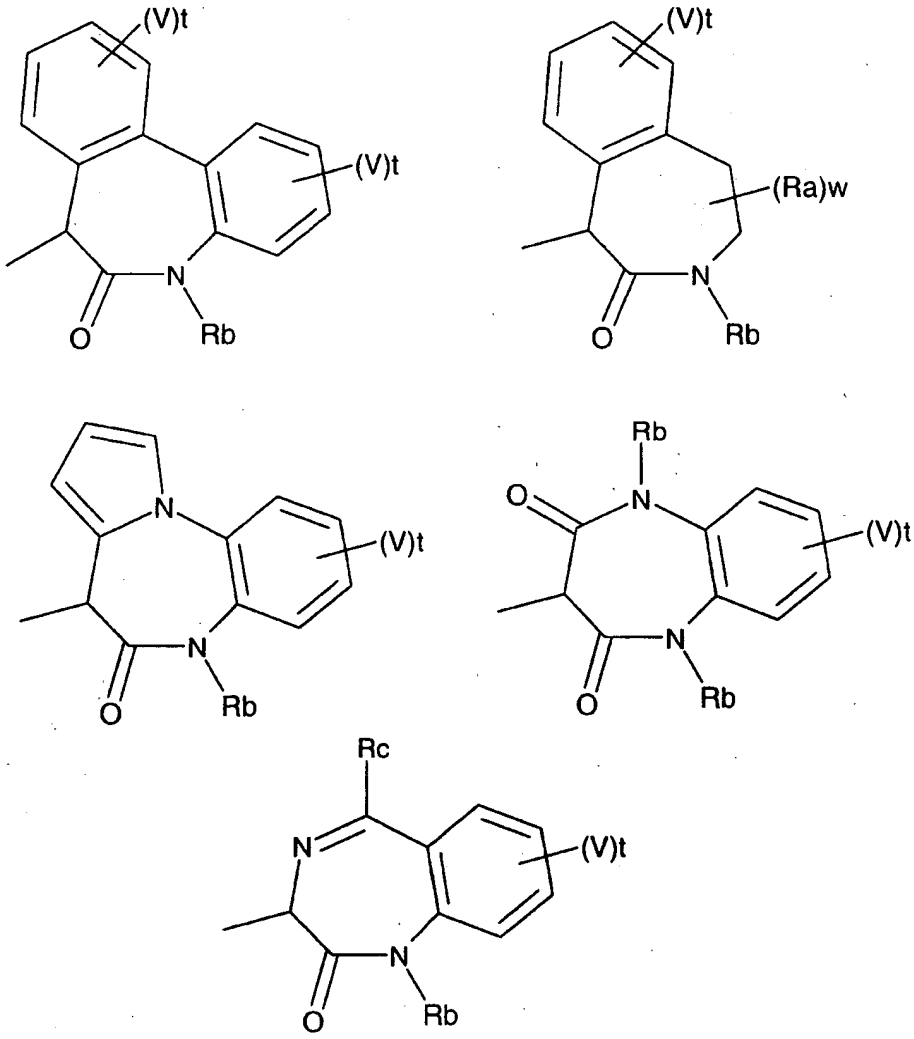
12. A compound of Claim 1 wherein Z is $-CH_2-$.

13. A compound of Claim 1 wherein Z is $-CH(OH)-$.

14. A compound of Claim 1 wherein t is 0.

15. A compound of Claim 1 wherein w is 0.

16. A compound of Claim 1 wherein W is a cyclic group selected from the group consisting of



wherein

Rb is selected from the group consisting of hydrogen, alkyl and aryl;

Rc is selected from the group consisting of alkyl, and aryl;

t is 0; and

w is 0.

17. A compound of Claim 4 wherein Z is $-CH_2-$.

18. A compound of Claim 4 wherein Z is -CH(OH)-.

19. A compound of Claim 6 wherein Z is $-CH_2-$.

20. A compound of Claim 6 wherein Z is -CH(OH)-.

21. A compound according to Claim 1 wherein the compound is 5-(S)-N'-(N''-((S)-2-Hydroxy-3-methylbutyrl)-(R)-β-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one.

22. A compound according to Claim 1 wherein the compound is 5-(S)-N'-(N''-((S)-2-Hydroxy-3-methylbutyrl)-(S)-β-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one.

23. A compound according to Claim 1 wherein the compound is 5-(S)-N'-(N''-((S)-3,5-difluorophenyl-α-hydroxyacetyl)-(S)-β-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one.

24. A compound according to Claim 1 wherein the compound is 5-(S)-N'-(N''-((S)-3,5-difluorophenyl-α-hydroxyacetyl)-(R)-β-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one.

25. A compound according to Claim 1 wherein the compound is 5-(S)-N'-(N''-((S)-2-Hydroxy-3-methylbutyrl)-(R)-α-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one.

26. A compound according to Claim 1 wherein the compound is 5-(S)-N'-(N''-((S)-2-Hydroxy-3-methylbutyrl)-(S)-α-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one.

27. A compound according to Claim 1 wherein the compound is 5-(S)-N'-(N''-((S)-3,5-difluorophenyl-α-hydroxyacetyl)-(R)-α-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one.

28. A compound according to Claim 1 wherein the compound is 5-(S)-N'-(N''-((S)-3,5-difluorophenyl-α-hydroxyacetyl)-(S)-α-methyl-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one.

29. A compound according to Claim 1 wherein the compound is 5-N'-(N''-(3,5-difluorophenylacetyl)-

β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one.

30. A compound according to Claim 1 wherein the compound is 5-N'-(N''-(1-(thien-2-yl)acetyl)-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one.

31. A compound according to Claim 1 wherein the compound is 5-N'-(N''-(3-methylbutyrl)-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one.

32. A compound according to Claim 1 wherein the compound is 5-N'-(N''-(phenylacetyl)-β-alaninyl)-amino-7-methyl-5,7-dihydro-6H-dibenz(b,d)azepin-6-one.

33. A pharmaceutical composition comprising a compound according to Claim 1 and a pharmaceutically acceptable diluent.

34. A method for inhibiting β-amyloid peptide release and/or its synthesis in a cell which method comprises administering to such a cell an effective amount of a compound according to Claim 1.

35. A method for preventing the onset of Alzheimer's disease in a human patient at risk for developing Alzheimer's disease which method comprises administering to said patient an effective amount of a compound according to Claim 1.

36. A method for treating a human patient with Alzheimer's disease which method comprises administering to said patient an effective amount of a compound according to Claim 1.